Short Communication

Studies on Fatty Acid Elongation and Desaturation in Rat Liver Phospholipids *in vivo**

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The fatty acid composition of membrane phospholipids exerts a major influence on membrane structure and function. Membrane stability, fluidity and permeability and the modulation of enzyme proteins are among the properties influenced by phospholipid fatty acids.[1] Changes in the composition and metabolism of these entities often reflect disturbances in the normal metabolism of the living cell. Studies on fatty acid elongation and desaturation have demonstrated that linoleic acid with unsaturation at positions 6 and 9 may be synthesized in eucaryotic cells. The precursor for longer polyunsaturated fatty acids is, however, the essential fatty acid linoleic acid (with double bonds at positions 9 and 12) or, in some cases, linolenic acid (with unsaturation at positions 9, 12 and 15).

Gas chromatographic procedures for the separation and quantitation of all fatty acids found in phospholipids are available today. This approach is, however, less suitable for monitoring the radioactive labeling of fatty acids. The extent of *in vivo* labeling using radioactive fatty acids is usually too small to permit quantitation of the radioactivity after derivatization and gas chromatography.

In recent years fluorescent probes which react with carboxyl groups have been developed, making possible appropriate derivatization of a number of compounds for high performance liquid chromatography (HPLC).[3] The great advantage of this approach is the ability to detect separated peaks with high sensitivity and at the same time to collect the eluate for scintillation counting.

The method developed in the work described here requires the separation of individual phospholipids before hydrolysis. We have used 9-anthryldiazomethane (ADAM) as reagent,[4] which reacts quantitatively with all fatty acids within 30 min. Employing a C-18 reversed-phase column and an appropriate gradient, the fatty acids could be isolated with base-line separation with few exceptions. Two additional chromatographic steps were required to obtain complete resolution: the original peak containing 14:0 and 16:1 fatty acids was subjected to chromatography on a C-8 column using isocratic elution. In addition, two incompletely resolved peaks containing, on the one hand, 18:3 and 22:6 and, on the other, 16:0 and 18:1 fatty acids were mixed and rechromatographed on a C-8 column using both isocratic and gradient systems. These steps together resulted in complete resolution of all fatty acids present in liver phospholipids, and scintillation counting of the eluate allowed determination of the specific radioactivity after *in vivo* administration of [^{14}C]palmitic acid.

A large variety of fatty acids were present in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) isolated from liver mitochondria and microsomes (Table 1). The major components were 16:0, 18:0, 18:1, 18:2, 20:4 and 22:6. Both 16:1 and, in particular, 20:3 were present in

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Table 1. Fatty acid composition (% of total) of liver mitochondrial and microsomal phospholipids. The values are the means of 5 experiments. PC = phosphatidylcholine, PE = phosphatidylethanolamine.

Fatty acid	Mitochondria		Microsomes	
	PC	PE	PC	PE
14:0	0.3	0.4	0.4	0.4
16:0	24.5	20.4	22.2	20.4
16:1	5.8	1.6	5.7	1.6
18:0	16.0	14.9	13.2	14.9
18:1	6.1	4.2	6.9	4.2
18:2	17.7	17.5	19.4	17.5
20:0	0.7	0.2	0.6	0.2
20:1	0.4	0.3	0.4	0.3
20:3	2.4	1.0	2.8	1.0
20:4	12.7	13.6	15.1	13.6
22:0	0.7	0.2	0.6	0.2
22:3	1.2	0.6	0.9	0.6
22:6	10.6	24.8	10.8	24.8
24:0	0.9	0.3	0.8	0.3

considerably larger amounts in PC than in PE of both fractions. In other respects the fatty acid compositions of these two phospholipids were similar.

[^{14}C]Palmitic acid was injected intraperitoneally and incorporation of this compound into membrane phospholipids was monitored (Table 2). Labeling of the various fatty acid fractions of both PC and PE was high after 30 min, at which point in time the specific radioactivity was considerably higher in microsomes than in mitochondria. This finding is consistent with localization of phospholipid biosynthesis in the endoplasmic reticulum.[5] Most of the palmitic acid is elongated and desaturated already after 30 min, and 70–80 % of the radioactivity is associated with linoleic acid. The ratio of microsomal to mitochondrial labeling for all fatty acids decreased in the interval between 30 and 90 min after injection. This fact also provides evidence for time-dependent transport of phospholipids from the endoplasmic reticulum to mitochondria. The picture is influenced by the exchange reaction known to occur for some fatty acids.[6] The relative increase in labeling of palmitic acid and the decreased radioactivity in linoleic acid are in agreement with the rapid rate of exchange of the latter fatty acid in liver.[7]

Since [^{14}C]palmitic acid was injected as precursor, it was quite natural to recover radioactivity in this fatty acid both in PC and PE of the membranes. However, 16:0 contained no more than 20 % of the total radioactivity (Table 3). About 5–10 % of the radioactivity appeared in 16:1, and the remainder, i.e. 70 %, was associated with the condensation product containing 18 carbon atoms. About 5–15 % of the radioactivity was recovered in 18:0, a few per cent in 18:1, while most of the activity, which is more than half of the total, was associated with 18:2. Under our conditions no radioactivity was recovered in longer fatty acids.

These experiments demonstrate that the fluorescence labeling procedure followed by appropriate separation on HPLC is effective for the

Table 2. Radioactivity (cpm per μg Pi) in the total fatty acid fraction of mitrochondrial and microsomal phospholipids after *in vivo* labeling with [^{14}C]palmitic acid. The values are the means of 4 experiments. PC = phosphatidylcholine, PE = phosphatidylethanolamine.

		Incorporation time		
		30 min	60 min	90 min
Mitochondria				
PC	16:0	133	36	15
	16:1	28	23	3
	18:0	19	11	11
	18:1	9	3	2
	18:2	759	132	45
PE	16:0	94	37	21
	16:1	63	12	4
	18:0	39	15	6
	18:1	16	3	2
	18:2	575	101	23
Microsomes				
PC	16:0	183	44	28
	16:1	61	41	6
	18:0	138	50	33
	18:1	31	22	10
	18:2	1116	186	48
PE	16:0	249	67	31
	16:1	47	9	5
	18:0	62	46	13
	18:1	47	9	2
	18:2	1150	173	45

Table 3. Distribution of radioactivity (% of total) in various fatty acids of liver mitochondrial and microsomal phospholipids after *in vivo* labeling. The incorporation time was 60 min. The values are the means of 4 experiments. PC = phosphatidylcholine, PE = phosphatidylethanolamine.

	Fatty acid				
	16:0	16:1	18:0	18:1	18:2
Mitochondria					
PC	17.6	11.4	5.4	1.4	64.2
PE	22.3	7.2	8.8	1.5	60.2
Microsomes					
PC	12.9	12.0	14.5	6.4	54.2
PE	22.0	3.1	15.2	2.8	56.9

isolation and quantitation of all fatty acids present in liver membrane phospholipids. Since the fatty acids are recovered quantitatively, the radioactivity incorporated under *in vivo* conditions is sufficient for the determination of specific radioactivity. The [^{14}C]palmitic acid injected is partially consumed by β-oxidation and is incorporated to a smaller extent into phospholipids in unchanged form. Part of the palmitic acid is subjected to desaturation or condensation to yield 18-carbon fatty acids with or without desaturation. With this approach it will be possible to study the metabolic transformations of various injected labeled fatty acids and to establish their role in the biosynthesis of various lipids containing fatty acids residues.

Experimental

Male rats weighing 60 g were injected intraperitoneally with 100 μCi [U-^{14}C]palmitic acid (928 mCi mmol^{-1}, Amersham). Mitochondria and microsomes were prepared from the liver homogenate as described earlier.[8] The lipids were extracted with $CHCl_3$/MeOH/H_2O (2:1:0.2) and individual phospholipids were separated by two-dimensional thin-layer chromatography.[9] The isolated phospholipids were subjected to alkaline hydrolysis and the extracted fatty acids were derivatized with 9-anthryldiazomethane (ADAM) reagent. HPLC separation was performed on a C-18 resolve column (Waters) using gradient program No. 7 (Waters 660 solvent program) with acetonitrile/H_2O (90:10) in pump system A and acetonitrile/tetrahydrofuran (60:40) in pump system B. Peak 4 was subjected to rechromatography on a C-8 column (Lichrosorb RP-8, Merck) using isocratic elution with acetonitrile/H_2O (88:12) (separation of 14:0 and 16:1). Peaks 1+2 and 7+8 from the first chromatography were collected, mixed and re-run on a C-8 column. The gradient used was No. 6 (Waters 660 solvent program), and acetonitrile/MeOH/H_2O (40:40:20) was used in pump system A and isopropanol/acetonitrile (70:30) in pump system B (separation of 16:0, 18:1, 18:3 and 22:6). The individual fatty acids were collected and their radioactivity determined by scintillation counting. They were identified by comparison with standards and, in some cases, by mass spectrometry.

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