INCORPORATION OF ^{32}P-PHOSPHATE INTO PHOSPHATIDES OF RAT LIVER MITOCHONDRIA IN VIVO AND IN VITRO

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1. Introduction

Recently the occurrence of the enzymes necessary for the synthesis of lecithin could be demonstrated in the outer membrane of rat liver mitochondria [1]. Also the incorporation of precursors into phosphatides of the outer mitochondrial membrane was shown *in vitro* [2]. But the origin of phosphatides of the inner membrane, which represents about 80% of the total, remains to be demonstrated. *In vitro* experiments suggested a transfer of phosphatides from microsomes into the inner compartments of mitochondria [3], which is similar to the transfer of proteins [4] and cytochrome c [5]. This paper describes a more detailed analysis of the origin of mitochondrial phosphatides.

2. Methods

Rat liver mitochondria and microsomes were prepared as described [6]. The inner and outer membranes of mitochondria were prepared by the improved digitonin method of Schnaitman [7]. 12 mg/100 mg mitochondrial protein of digitonin was used. The low-speed sediment was sonicated in the mannit-medium and centrifuged for 1 h at 100,000 × *g*. Both the supernatants of the outer and inner membranes were again centrifuged for 1 h at 400,000 × *g*. The resulting supernatants were designated intracristae- and matrix-fraction.

The *in vitro* experiments were performed at 37° in a medium consisting of: 100 mM KCl, 4 mM $MgCl_2$, 40 mM Tris-HCl, pH 7.6, 1 mM ATP, 0.2 mM CTP, 5 mM a-ketoglutarate, 3 mg mitochondrial protein/ml and 40 μc ^{32}P-phosphate in a final volume of 5 ml. For the experiments *in vivo* 0.3–0.4 mc ^{32}P-phosphate in 0.9% NaCl were injected intraperitoneally (0.5 ml) into male rats of 190–210 g body weight. The specific activity of inorganic phosphate was assayed after extraction with isobutanol-benzene according to Lindberg and Ernster [8].

Extraction of phosphatides: Folch et al. [9]. Two dimensional thin-layer chromatography: Fleischer et al. [10]. Spots were scratched out, digested, taken up with water and equal parts were used for determination of inorganic phosphate [11] and ^{32}P-activity [12].

3. Results and discussion

After incubation with ^{32}P-phosphate for 20 min, mitochondria were separated into subfractions and the total content and specific activities of the individual phosphatides estimated as shown in table 1 *. In agreement with the results of Kaiser and Bygrave [2] and of Stoffel and Schiefer [1], all phosphatides had the highest specific activity in the outer membrane. Only LPC showed the highest specific activity in the intracristae-fraction. But also the percentage of LPC from total phosphatides was found much higher in the intracristae fraction, compared to the other fractions as shown in table 2.

* *Abbreviations:* PC = phosphatidylcholine, PE = phosphatidylethanolamine, PS = phosphatidylserine, PI = phosphatidylinositol, LPC = lysophosphatidylcholine, Card = cardiolipin.

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Table 1
Specific activities of phosphatides of mitochondrial subfractions; ^{32}P-phosphate for 20 min *in vitro*.

	Total phosphatide (mU moles phosphatide-phosphate/mg protein)	PC	PE	PS+PI	LPC
		(cpm X 100/mU moles phosphatide-P)			
Inner membrane	345	2.5	14	164	114
Matrix	35	0	5	120	0
Outer membrane	660	17	95	600	88
Intracristae	195	14	12	115	340

Table 2
Percentage of total phosphatides in subfractions of rat liver mitochondria.

	Inner membrane	Matrix	Outer membrane	Intra-cristae
PC	39	54	51	54
PE	39	30	34	27
PS+PI	10	5	9	11
Card	9	6	3	2
LPC	0.9	0.9	0.6	2.2

Fig. 1 demonstrates the kinetics of incorporation of ^{32}P-phosphate into phosphatides of rat liver fractions *in vivo*. Together with the specific activity of inorganic phosphate, time sequences of labeling of the phosphatides were observed, which were compatible with a precursor-product relationship between PE of microsomes and PE of mitochondria and between PE and PC. A similar time sequence of labeling was found for PE and PS from soluble and insoluble protein fractions of mitochondria as shown in fig. 2. Mitochondria labeled with ^{32}P-phosphate *in vivo* were sonicated in 0.3 M sucrose and centrifuged for 1 h at 400,000 × *g*. The phosphatides PE and PS extracted from the supernatant were higher labeled than those from the sediment. In contrast, the specific activity of PC was lower in the supernatant, and became labeled in both fractions only after 20 min. We conclude that at least a part of the mitochondrial PC is formed from PE (probably by methylation of the ethanolamine) which has been synthesized at the endoplasmic reticulum and transferred into the mitochondria.

Table 3 compares the incorporation rates of ^{32}P-

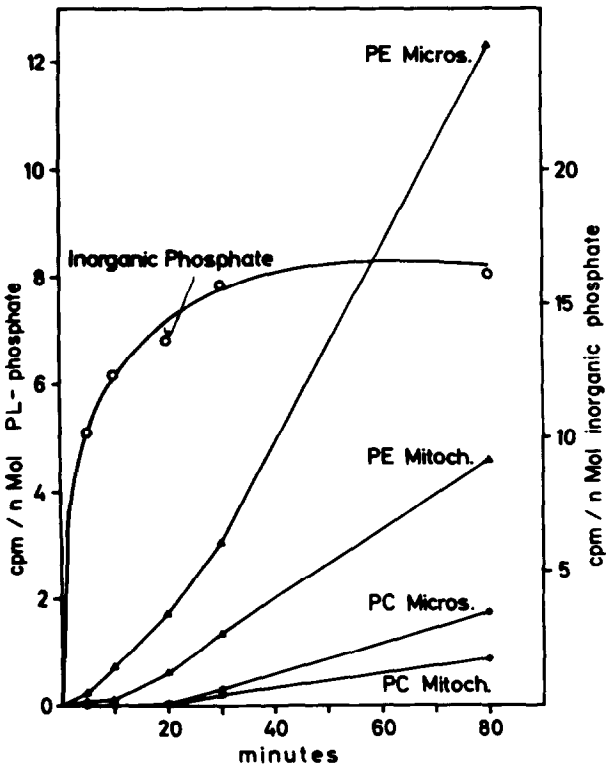

Fig. 1. Time-dependency of incorporation of ^{32}P-phosphate into phosphatides of rat liver *in vivo*.

phosphate *in vivo* and *in vitro*. The calculation was based on the specific activities of inorganic phosphate in the liver and in the incubation medium. For the *in vitro* experiment the rates between 0 and 10 min, and for the *in vivo* experiments 1/5 of the rates between 30 and 80 min are indicated. Whereas the rates for

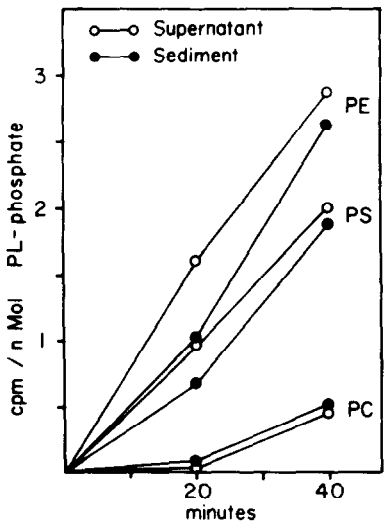

Fig. 2. Incorporation of ^{32}P-phosphate into phosphatides of the sediment and supernatant of sonicated rat liver mitochondria *in vivo*.

Table 3
Comparison of incorporation rates of ^{32}P-phosphate into phosphatides of rat liver mitochondria *in vivo* and *in vitro*.

	In vivo	*In vitro*
	(mmoles ^{32}P-phosphate/moles phosphatide-phosphate in 10 min)	
PC	8.1	(0.08)
PE	40	(0.29)
PS	29	4.9
PI	20	0.3
LPC	2.1	2.4

PC- and PE-synthesis *in vitro* may have been too low due to a deficiency of precursors, with PS which was rapidly labeled *in vitro*, the rate of synthesis was 6 times higher *in vivo*. Only LPC showed equal rates *in vivo* and *in vitro*, suggesting that labeling of this mitochondrial phosphatide occurs only within the mitochondria, possibly by a pathway which does not involve PC as an intermediate.

The results support the idea already presented in an earlier paper [13] that phosphatides parallel the transfer of proteins from microsomes into mitochondria. The mechanism of this transfer, possibly by means of a protein-phospholipid transferring unit, is still obscure and remains to be evaluated.

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