Two Pools of Microsomal Phosphatidylethanolamine Detected by the Use of Fluorescamine *

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The microsomal membranes of the liver are relatively rich in phospholipids. These membranes also play a central role in the cellular lipid metabolism, since the vast majority of the phospholipids are synthetized on their cytoplasmic surfaces.[1] The newly synthesized phospholipids have several routes of distribution. Part of them remain in the microsomes but the majority is partly transported to other membranes and partly transported into the lumen of the endoplasmic reticulum to be used as components of the secretory lipoproteins. Previous experiments have indicated that microsomal phosphatidylethanolamine (PE) may be distributed in a non-random fashion.[2] In this previous investigation it was suggested that PE – which makes up about 30 % of total microsomal phospholipids – is compartmentalized, that is, is present in the membrane in pools where this lipid exhibits different turnover. In order to approach this problem we have studied the interaction of PE with the monofunctional amino reagent fluorescamine (FLA).

Microsomal membranes are suitable for studies with FLA since the reagent freely penetrates these membranes and reacts instantaneously with PE, even at low temperatures. Isolated, washed total liver microsomes (5 mg) were incubated in a medium containing 50 mM phosphate buffer pH 7.8, 100 mM NaCl and FLA. The experiments were performed in the absence and presence of 0.25 and 0.05 % Triton X-100. The incubation was conducted at 20 °C for 15 s during continuous stirring. The reaction was quenched by addition of 100 μl 250 mM aniline. The microsomes were extracted with chloroform—methanol—water (2:1:0.2 v/v) and the free PE (PE_f) were separated from that part of PE which is covalently bound to FLA (PE_b) by thin-layer chromatography. When microsomes are incubated with the reagent at increasing concentrations of FLA the semi-logarithmic plot detects two pools (Fig. 1). The first pool is reacted with high efficiency while the second pool, which represents about 20 % of the total, is characterized by poor interaction even at high reagent concentrations. When the membrane barrier is eliminated by addition of 0.25 % Triton X-100, the second pool is abolished and all PE is rapidly reacted with FLA. It is known that lower Triton concentrations (0.05 %) eliminate membrane permeability for macromolecules without disrupting the membrane structure.[3] Significantly, this detergent concentration is insufficient in removing the second pool which demonstrates that this pool exists because of the structural property of the membrane itself and is not caused by limitation in membrane permeability.

The fatty acid (FA) composition of the two PE pools is shown in Table 1. A number of differences in distribution of total FA appears. In some cases the differences are very pronounced (2–4 fold). These FAs are represented by the 18:2, 20:4 and 22:6 populations. By using phospholipase A_2, we have also investigated the FA distributions at position 1 and 2. Some of the differences are very striking. The two main saturated FAs, 16:0 and 18:0, are found only at position 1 in the pool consisting of PE bound to FLA, while in the free pool they are also present at position 2. Most interestingly, we found that 18:1 is situated only at position 1 in the bound pool and only at position 2 in the free one. Linoleic acid (18:2) is distributed only at position 2 in the bound pool and at both positions in the free pool where its total amount exceeds 4 times that of the other pool. The differences described above are highly significant and indicate a chemical or a metabolic difference between the two established compartments. Since certain fatty acids are present at single and opposite locations in the two pools, the possibility arises that the location of the fatty acid on the

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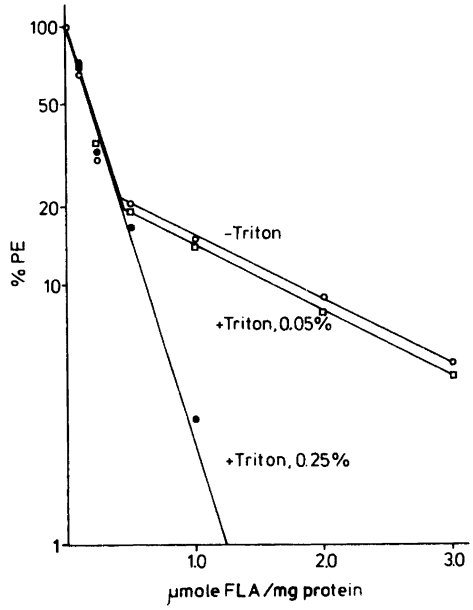

Fig. 1. Reaction of FLA with microsomal PE. Incubations are performed without detergent and in the presence of 0.05 or 0.25 % Triton X-100.

phospholipid molecule may target the lipid from the place of synthesis to the place of its final location.

Labeling of PE was performed by intraportal administration of [^{14}C]palmitic acid. Rats were injected with 60 kBq [^{14}C]palmitic acid – adsorbed to bovine serum albumin – into the portal vein at various time points before decapitation. The isolated liver microsomes were treated with FLA, PE was extracted and after separation of the free and the PE-bound pools, the specific radioactivity in the PE was determined. High specific labeling was initially

Table 1. Distribution of fatty acids in free PE and PE bound to FLA compartments. After incubation with FLA (1 μmol per mg protein), PE pools were separated and after hydrolysis and methylation the total fatty acid content was determined by capillary gas chromatography. The same lipids were also treated with phospholipase A_2 and the fatty acid content of the lysocompound was analyzed. The data given represent the mean value of 8 experiments, per cent of total.

Fatty acid	PE bound			PE free		
	PE	FA in pos. 1	FA in pos. 2	PE	FA in pos. 1	FA in pos. 2
15:0	0.7	1.0	0.4	3.1	4.0	2.3
16:0	18.5	36.0	1.0	22.9	34.0	11.8
17:1	0.5	0.2	0.8	0.7	0.2	1.2
17:1	0.9	–	1.8	1.9	3.9	–
18:0	25.1	49.6	0.6	26.0	42.5	9.5
18:1	5.7	11.5	–	7.7	1.5	14.0
18:2	3.6	–	7.2	14.0	10.0	18.0
20:3	0.5	1.0	–	3.5	5.1	1.9
20:4	22.1	–	44.2	12.3	–	24.6
20:5	0.8	–	1.6	0.1	–	0.2
22:5	1.7	–	3.4	0.5	–	1.0
22:6	19.8	–	39.7	7.5	–	15.0

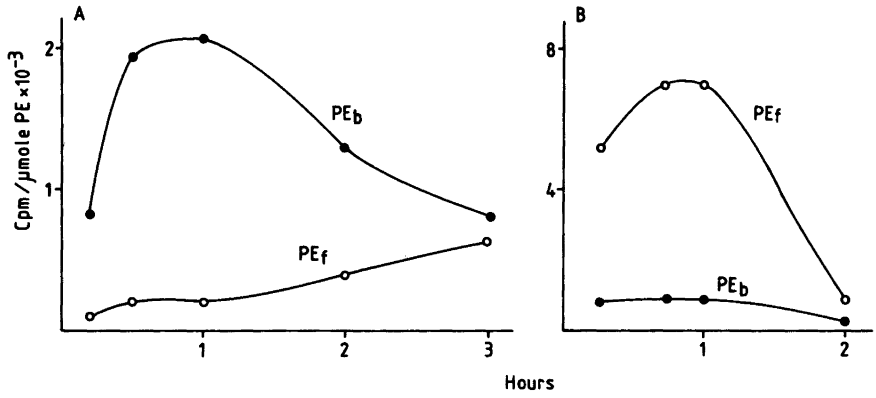

Fig. 2. In vivo incorporation of palmitic acid and PE into liver microsomes. A. *In vivo* injection of 60 kBq [^{14}C]palmitic acid into the portal vein. B. Injection of 5.6 kBq 1-palmitoyl-2-[1-^{14}C]palmitoyl PE liposomes into the portal vein. In both experiments 1 μmol FLA per mg protein was used in the incubations. PE_f=free phosphatidylethanolamine; PE_b=phosphatidylethanolamine covalently bound to fluorescamine.

obtained in the PE of the pool bound to FLA while the rest of the PE had very low labeling (Fig. 2A). Equilibration of the two pools required 3 h. In order to test the idea that the location of the fatty acid may have a targeting function, dipalmitoyl PE labeled at the 2 position was utilized (Fig. 2B). When the lipid (1-palmitoyl-2-[1-^{14}C]palmitoyl PE), in liposomes form, was injected into the portal vein, PE was taken up by the hepatocytes and the lipid could be detected also in the microsomal membrane as a constitutive component. The distribution of the injected lipid in the two microsomal compartments is highly significant. After 1 h the specific labeling in the pool, which is not reacted with FLA, exeeds 7 times that of the major pool. Another significant aspect of this labeling is that no equilibration of radioactivity between the two pools occurs during 2 h, which is in contrast to the experiment previously described with palmitic acid. This experiment shows that PE is taken up only into that pool which has a similar FA composition as the injected lipid. In this case only the unreacted pool has palmitic acid at the 2 position (see Table 1).

The described experiments demonstrate that PE in microsomal membranes is compartmentalized. It is not known why this occurs but the explanation may be probe inaccessibility to a pool with different organization such as hexagonal H_{II} phase.[4] The fatty acid composition of the two compartments of PE not only reflects quantitative but also pronounced qualitative differences. The results indicate that the fatty acid on the PE molecule may serve as a signal for targeting of the lipid from the site of synthesis to a final location in a specific pool.

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