Arrangement of Aminophospholipids in the Microsomal Membranes *

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The membranes of the endoplasmic reticulum isolated from rat liver are enriched in phospholipids. The two aminophospholipids are phosphatidylethanolamine (PE) and phosphatidylserine (PS), which constitute 25 and 7 %, respectively, of the total phospholipid. Using model membranes, it was postulated that an intensive lateral mobility occurs, while transmembrane movement is absent.[1] On the other hand, the findings on protein-immobilized lipid regions impose a limitation on the lateral movement. In addition, incubation of liposomes with microsomes in the presence of exchange proteins led to a complete exchange of phospholipids, which would suggest free transmembrane mobility.[2] Clearly, experiments with model membranes are not identical with those performed under *in vivo* conditions and incubation of microsomes *in vitro* may result in rearrangement of the membrane structure.

Table 1. Cross-linking of aminolipids in the microsomal membranes with 1,5-difluoro-2,4-dinitrobenzene. The incubation medium contained 40 mM NaCl, 120 mM $NaHCO_3$, pH 8.5, 8 mM EDTA, 0.25 M sucrose and 10 mg microsomal protein in a final volume of 4 ml. After incubation for 17 h at 20 °C the medium was diluted with 5 ml cold 0.25 M sucrose and centrifuged at 105 000 *g* for 45 min. The pellet was extracted with chloroform–methanol (2:1) and the individual dinitrobenzene derivatives were isolated by thin layer chromatography.[3] PE, phosphatidylethanolamine, PS, phosphatidylserine; FDNP, fluoro-2,4-dinitrophenyl; DNP, 2,4-dinitrophenyl.

	% of total
PE as FDNP–PE	26
PE–DNP–PE	9
PE–DNP–PS	2
PE–DNP–Protein	63
PS as FDNP–PS	39
PS–DNP–PS	24
PS–DNP–PE	6
PS–DNP–Protein	31

Microsomal vesicles were incubated with 1,5-difluoro-2,4-dinitrobenzene (DFDNB), which is a bifunctional reagent reacting with amino groups of lipids and proteins. Under the conditions used, microsomal membranes are completely permeable to this reagent and, consequently, interaction with all amino lipids can occur. Using total microsomes 26 % of the PE is recovered as monomer; the amount of dimer and the portion cross-linked to PS is very low; and the majority of this lipid is cross-linked to protein (Table 1).

The situation with PS is different. More than 60 % of this lipid is in monomer or dimer form and only about 30 % is cross-linked to protein. Thus, the reaction pattern of the two microsomal aminolipids with DFDNB indicates a different distribution in the membrane.

The validity of these cross-linking experiments was investigated using titration with the reagent. At a concentration of 0.75 mM, as used above, all microsomal PE reacts with DFDNB and at this concentration a maximal cross-linking to protein is obtained (Fig. 1). As expected, an increased concentration of the reagent results in competition for the aminophospholipids available and gives predominantly monomers. Various types of control experiments demonstrated that the results are not influenced by the phospholipid composition of membranes or by the phospholipid/protein ratio. In inner mitochondrial membranes, where the PS concentration is lower in relation to PE, only 33 % of the PE is cross-linked to protein (not shown in table). In erythrocytes, where the PE/PS ratio is similar to that of microsomes, only 4 % of the PE can be cross-linked to protein.[3]

The finding that PE interacts in several ways with DFDNB raises the possibility that this lipid may occur in different compartments within the membrane. If this is the case, it is possible to investigate the movement from one compartment to the other. Rats were labeled *in vivo* with [^{3}H]-glycerol and after various incorporation periods liver microsomes were prepared. The membranes were incubated with 0.75 mM DFDNB and the labeling pattern in the monomer and the protein-bound form of PE was investigated (Fig. 2). Even after times as short as 15 min, a large amount of radioactivity is present in the monomer form and at this time almost no label can be detected in the

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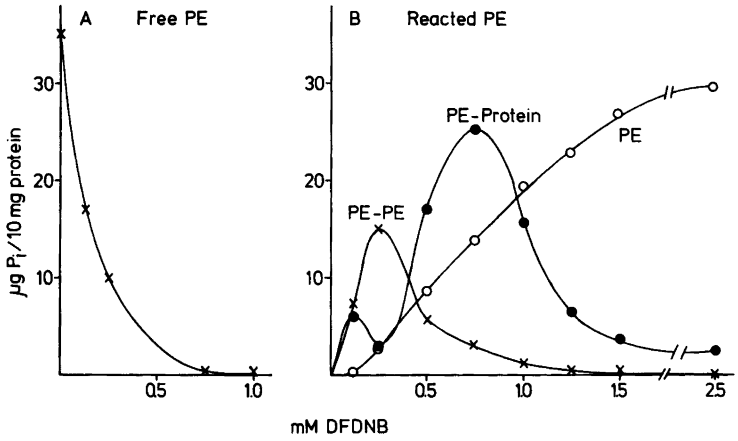

Fig. 1. Reaction of phosphatidylethanolamine with 1,5-difluoro-2,4-dinitrobenzene (DFDNB). Microsomes were incubated with various concentrations of DFDNB and the individual dinitrobenzene derivates were isolated by thin layer chromatography. PE, phosphatidylethanolamine.

protein-bound form. Peak incorporation in the latter occurs with a delay of about 1 h. It appears that the monomer form represents newly synthesized molecules of PE, which should be localized close to the cytoplasmic surface where the enzymes involved in its biosynthesis are found. From this initial location PE is probably redistributed to the protein-bound form in a time-dependent process.

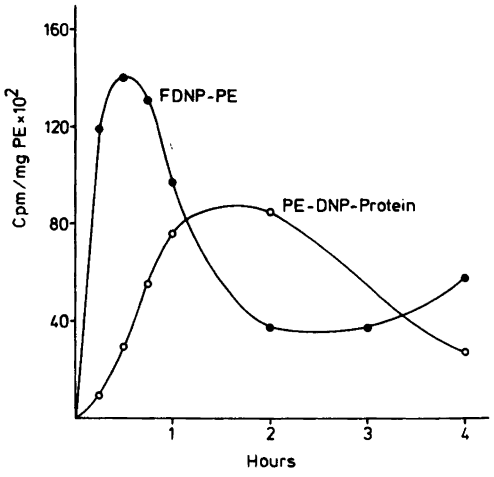

Fig. 2. Distribution of [^{3}H]glycerol labeled phosphatidylethanolamine after DFDNB treatment. Rats were injected through the portal vein with 5.55 MBq [^{3}H]glycerol and after various time-points liver microsomes were isolated, treated with DFDNB and the lipids subjected to chromatography. Radioactivity and phosphate in the individual spots were determined. PE, phosphatidylethanolamine; FDNP, fluoro-2,4-dinitrophenyl; DNP, 2,4-dinitrophenyl.

The possibility that PE is associated with the enzymes catalyzing hydroxylation reactions was also investigated, since such an association is continuously discussed in the literature.[4] Phenobarbital treatment increases the NADPH-cytochrome *c* reductase and cytochrome P-450 content of microsomes severalfold, while methylcholanthrene treatment increases preferentially another type of cytochrome P-450. Cross-linking studies were performed on liver microsomes prepared from induced rats and the pattern was compared with that of the control. Both inducers resulted in microsomes, where the cross-linking pattern exhibited a moderate change, *i.e.*, about 15 % decrease of the protein-bound form of PE. This pattern does not support the preferential association of PE with hydroxylating enzymes. Rough microsomes were also subfractionated using rate-differential centrifugation to yield fractions enriched either in electron transport enzymes or phosphatases. Again, the enzyme heterogeneity was not paralleled by an uneven distribution of phospholipids.

It appears that the majority of PE in microsomal membranes is present in a protein-bound form, which represents a compartment separate from that of the non-protein bound form. Aminophospholipids exhibit a restricted mobility and obviously do not participate as components of a completely fluid system. The induction experiments do not favor

the idea that these lipids are the major factors in immobilization of hydroxylating enzymes.

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