NET TRANSFER OF PHOSPHATIDYLINOSITOL FROM MICROSOMES AND MITOCHONDRIA TO LIPOSOMES CATALYZED BY THE EXCHANGE PROTEIN FROM RAT LIVER

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1. Introduction

It is now well established that an exchange of phospholipids can occur between intracellular organellae [1–6] (for review see [7]). This exchange is catalyzed by special exchange proteins found in the cytosol of several tissues [8–15]. These proteins seem to be rather specific toward individual phospholipids [9, 11–13, 15] but unspecific with respect to membranes, i.e. they can catalyze the exchange of phospholipids between different biological as well as artificial membraneous structures, e.g. liposomes [16–19].

The exchange protein for PC* contains one molecule of PC per molecule of protein [7,17]. Kagawa et al. [20] provided an indirect evidence for a net transfer of PC from liposomes to reconstituted energy-coupling vesicles, as mediated by the exchange protein from beef heart, and Ehnholm and Zilversmit [11] showed a unidirectional transfer of sphingomyelin. Recently, Wirtz and his colleagues [21,22] have directly demonstrated a net transfer of PI between microsomes and liposomes catalyzed by the transfer protein isolated by these authors from beef brain [15]. Independently, we have briefly reported [23] on a net transfer of PI from microsomes and mitochondria to liposomes lacking this phospholipid as catalyzed by the transfer protein(s) from rat liver. The present paper describes these studies in more detail.

* *Abbreviations:* PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

2. Materials and methods

Mitochondria, microsomes and the soluble cytoplasmic fraction were isolated from rat livers by conventional methods [24]. Microsomes were thereafter treated with $CaCl_2$ according to Kamath and Rubin [25]. This treatment enabled their sedimentation by low speed centrifugation which was essential for their separation from liposomes. Liposomes were prepared from PC containing 4 mol% phosphatidic acid as described by Demel et al. [26]. PC used for this purpose had been extracted from rat liver, separated from other phospholipids by thin-layer chromatography in the system of Wagner et al. [27] and then further purified by two-dimensional thin-layer chromatography according to Rouser et al. [28]. Phosphatidylinositol (from beef brain, Folch Fraction I) and phosphatidic acid (from egg lecithin) were commercial products of Koch-Light Laboratories (Colnbrook, England).

In vivo labelling of mitochondrial and microsomal phospholipids was performed by injecting the rats with [^{32}P] phosphate 20 hr before killing [6]. In vitro labelling of microsomal phosphatidylinositol was obtained by preincubating microsomes with myo-[^{3}H] inositol in the presence of *sn*-glycerol 3-phosphate, fatty acids, ATP and CTP as described by Strunecká and Zborowski [29]. When microsomes labelled in proteins and phosphatidylinositol had to be used, rats were first injected with [^{14}C] leucine 1 hr prior to killing and then the isolated microsomes were preincubated with [^{3}H] inositol.

Exchange and transfer of phospholipids were

measured as follows. Labelled microsomes pretreated with $CaCl_2$ or mitochondria were incubated with liposomes in the presence of the cytoplasmic fraction at 30°C in the medium containing 250 mM sucrose, 1 mM EDTA and 2 mM Tris–HCl, pH 7.4. The reaction was terminated by sedimenting mitochondria or $CaCl_2$-pretreated microsomes at 15 000 *g* during 10 min. The liposomes and proteins remaining in the supernatant were precipitated with 10% trichloroacetic acid and the lipids were extracted from the precipitate with a mixture of chloroform and methanol (2:1, v/v) and reextracted with petroleum ether–chloroform (1:1, v/v). The extract was measured for radioactivity by scintillation counting. In case of ^{32}P labelling, individual phospholipids were separated by thin-layer chromatography [27,30], scraped off and counted by Čerenkov radiation [31].

Rotenone-insensitive NADH-cytochrome *c* reductase, used as marker for the outer mitochondrial membrane, was measured according to Sottocasa et al. [32]. Protein was determined by the biuret method [33].

3. Results and discussion

When microsomes pre-labelled in vivo with ^{32}P were incubated with liposomes a time-dependent transfer of the label to liposomes was observed (fig.1). Analysis of labelled phospholipids remaining in the

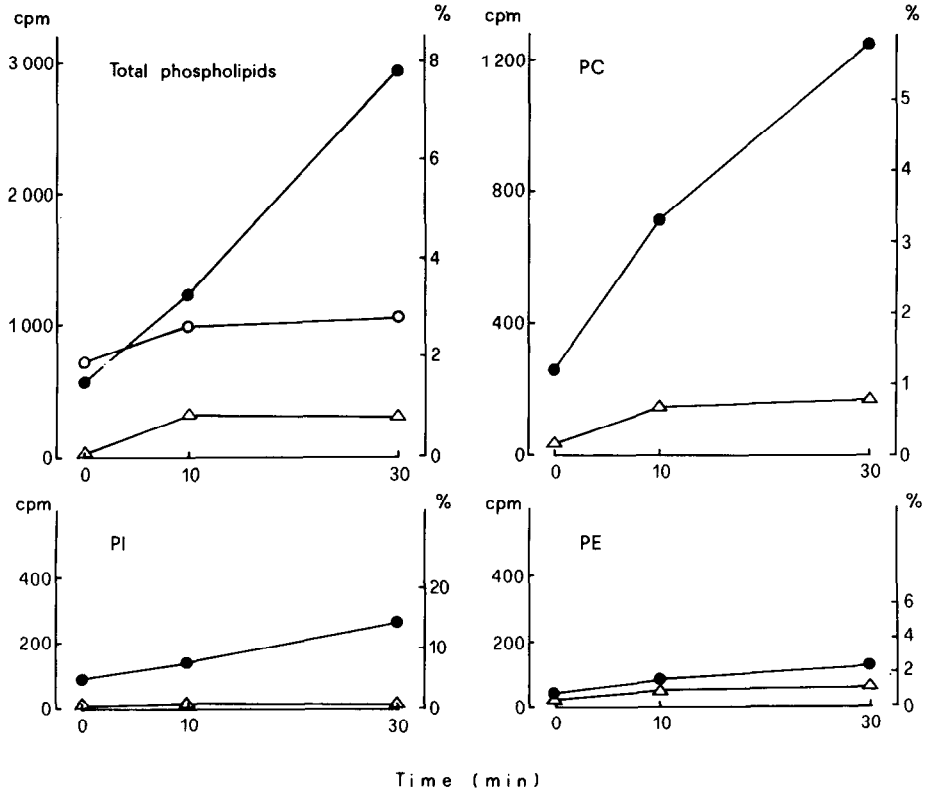

Fig.1. Exchange and transport of phospholipids between microsomes and liposomes. Ca^{2+}-treated microsomes (10.3 mg protein) containing ^{32}P-labelled phospholipids (PC, 21 600 counts/min; PE, 5660 counts/min; and PI, 1840 counts/min) were incubated with liposomes prepared from PC and phosphatidic acid (0.8 μmol phospholipid phosphorus per sample). Total vol was 2.3 ml. The amount of phospholipids found in the supernatant after sedimenting microsomes is expressed as counts/min ^{32}P (left oridinates) and as percentage of total or individual phospholipids initially present in microsomes (right ordinates). (●———●) Complete system (15.8 mg protein of the cytoplasmic fraction present); (○———○) cytoplasmic fraction omitted; (△———△) cytoplasmic fraction present but liposomes omitted.

supernatant after sedimenting microsomes revealed that PC, PI and, to a lower extent, PE were transferred. Omission of the cytoplasmic fraction greatly reduced the amount of the label transferred (see fig.1, total phospholipids). Omission of liposomes also drastically reduced the amount of labelled phospholipids released from microsomes, indicating that liposomes were the main acceptor site. Since liposomes initially contained no PI, this experiment points to a unidirectional net transfer of this phospholipid from microsomes to liposomes.

Similar results were obtained when microsomes were replaced by ^{32}P-labelled mitochondria. The activity of rotenone-insensitive NADH-cytochrome *c* reductase remaining in the post-mitochondrial supernatant was negligible and did not change during the incubation, thus indicating that labelling of liposomal PI was not due to contamination by fragments of the outer mitochondrial membrane.

A more conclusive evidence for unidirectional transfer of PI is provided by experiments in which microsomes specifically labelled in PI by in vitro preincubation with [^{3}H]inositol were used. Fig. 2 shows the dependence of [^{3}H]PI transfer on the amount of liposomes used.

To ascertain whether the labelling of the liposomal-cytoplasmic fraction was due to the transfer of PI or to contamination by non-sedimenting microsomal fragments, doubly labelled microsomes were used

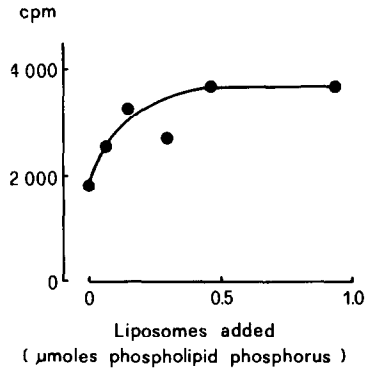

Fig.2. Dependence of phosphatidylinositol transport on liposome content. Ca^{2+}-treated microsomes (3.0 mg protein) containing [^{3}H] PI (17 000 counts/min) were incubated with liposomes in the presence of the cytoplasmic fraction (16.0 mg protein). Time of incubation 30 min.

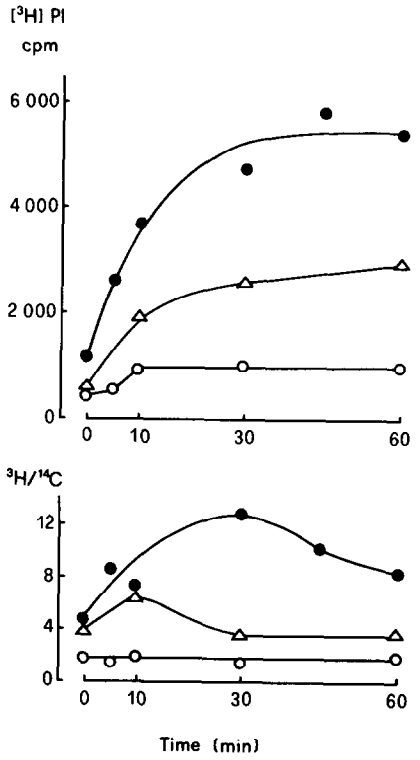

Fig.3. Transport of [^{3}H]phosphatidylinositol from microsomes to liposomes. Doubly labelled Ca^{2+}-treated microsomes 1.74 mg protein) containing 14 580 counts/min [^{3}H]PI and 4500 counts/min [^{14}C] proteins (^{3}H/^{14}C ratio of 3.24) were incubated with liposomes (0.8 μmol phospholipid phosphorus) prepared from PC and phosphatidic acid and with 21.5 mg protein of the cytoplasmic fraction in total volume of 2.8 ml. Radioactivity of ^{3}H and ^{14}C in the post-microsomal supernatant was measured without lipid extraction. (●———●) Complete system; (○———○) cytoplasmic fraction omitted; (△———△) cytoplasmic fraction present but liposomes omitted.

(fig. 3). Here again a cytoplasmic fraction-dependent and liposome-dependent transfer of [^{3}H]PI from microsomes to the post-microsomal supernatant is clearly visible. Moreover, the ^{3}H/^{14}C ratio in the post-microsomal supernatant substantially increases during first 30 min of incubation and only in samples where both liposomes and the cytoplasmic fraction are present. This indicates that, at least during initial 30 min, the transfer of [^{3}H]PI is faster than the solubilization of microsomal [^{14}C]proteins and points to a true unidirectional transfer of PI.

Harvey et al. [22] have recently shown that the transfer of PI from microsomes to liposomes, catalyzed by the transfer protein from beef brain, depends on phospholipid composition of the recipient liposomes and is inhibited by the presence of PI or phosphatidic acid therein. A similar observation was also made in the present study with respect to transfer protein(s) from rat liver. In our hands the transfer of [^{3}H]PI was diminished to 73% and 85% in two independent experiments when the liposomes contained 10 mol% PI. The conclusion may therefore be drawn that the presence of PI in liposomes is not only non-obligatory for the transfer to occur but, on the contrary, it may have an inhibitory effect. However, the present data do not allow us to decide whether the transport of a PI molecule from microsomes to liposomes is or is not accompanied by a concomitant transfer of a molecule of PC in the opposite direction.

The discovery of unidirectional transfer of PI and possibly of other phospholipids contributes to a better understanding of biogenesis and repair of biological membranes.

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