EXCHANGE OF DIFFERENT PHOSPHATIDYLCHOLINE MOLECULAR SPECIES BY PHOSPHOLIPID EXCHANGE PROTEIN OF RAT LIVER

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1. Introduction

In 1968 Wirtz and Zilversmit [1] described the stimulation of phospholipid exchange between mitochondria and microsomes by a protein of rat liver postmicrosomal supernatant. Following this, phospholipid exchange protein was characterised [2–4] and the exchange mechanism studied [5,6]. It was demonstrated that separate proteins might be involved in the protein-mediated exchange of different PL* [7,8]. Therefore it can be concluded that a series of PLEP exists each of which have different activities with respect to the exchange of individual classes of PL [9–11]. The question now is how does the activity of each exchange protein relate to the final PL composition of suitable acceptor membrane and does the exchange process have to be envisaged as a control mechanism of the lipid composition of cellular membranes?

Due to our interest in the fatty acid pattern of phosphatidylcholine and its regulation in biological membranes [12] we have undertaken an examination of the protein-mediated exchange of different PC molecular species with respect to fatty acids in positions 1 and 2 of PC.

**Abbreviations*: PL phospholipid, PC phosphatidylcholine, PLEP phospholipid exchange protein.
DPPC 1,2-dipalmitoyl-PC PPC 1-acyl-2-palmitoyl-PC
DSPC 1,2-distearoyl -PC SPC 1-acyl-2-stearoyl -PC
DOPC 1,2-dioleyl -PC OPC 1-acyl-2-oleyl -PC
DLPC 1,2-dilinoleyl -PC LPC 2-acyl-2-linoleyl -PC
PPO 2,5-diphenyloxazol, POPOP 1,4-bis(2-(5-phenyl)-oxazolyl)-oxazol

2. Materials and methods

PC exchange activity was determined as described previously [13], by measuring the exchange of PC between radioactively labeled liposomes and rat liver mitochondria after 60 min. Rat liver mitochondria were prepared according to Liese et al. [14]. The delipidisation of the mitochondria was performed with aqueous acetone [15]. The PL content of the lipid-extracted mitochondria was 2 nmol P_i/mg protein (40% PC). Normal mitochondria contained 220 nmol P_i/mg protein. A purification of PLEP was not undertaken. As exchange protein the pH 5.1-supernatant of rat liver according to Wirtz and Zilversmit [16] was used.

The following ^{14}C and ^{3}H labeled PC molecular species were synthesized as described recently: DPPC, DSPC, DOPC, DLPC, PPC, SPC, OPC, LPC [17,18]. The specific activities ranged between 2×10^4 and 10^6 cpm. The radioactivity was determined with a liquid scintillation counter, Wallac 81 000, LKB, Stockholm, using 8 ml scintillation solution (4 g PPO and 40 mg POPOP/980 ml toluene and 20 ml ethanol). The liposomes were made by ultrasonication in 10 ml incubation buffer (250 mmol/l sucrose, 10 mmol/l Tris–HCl, 1 mmol/l EDTA, pH 7.3) at 20 KHz for 3 min with a UD 125, Schöller-Schall, Frankfurt/M. at a temperature above the phase transition temperature. Liposomes were characterised by fractionation on Sepharose 4B [19]. P_i was determined as described by Rouser and Fleischer [20], the protein content according to Gornall et al. [21]. We have distinguished between a passive exchange as an increase of radioactivity without pH 5.1-supernatant and a protein-mediated active one

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in the presence of exchange protein corrected for the passive exchange rate. All results are the mean values of double or manifold determinations.

3. Results and discussion

As shown in fig.1 and 2 the single PC molecular species were exchanged to different extents according to the nature of the fatty acids in position 2. The exchange rate curves in relation to the PLEP content of the incubation mixture allow one to differentiate

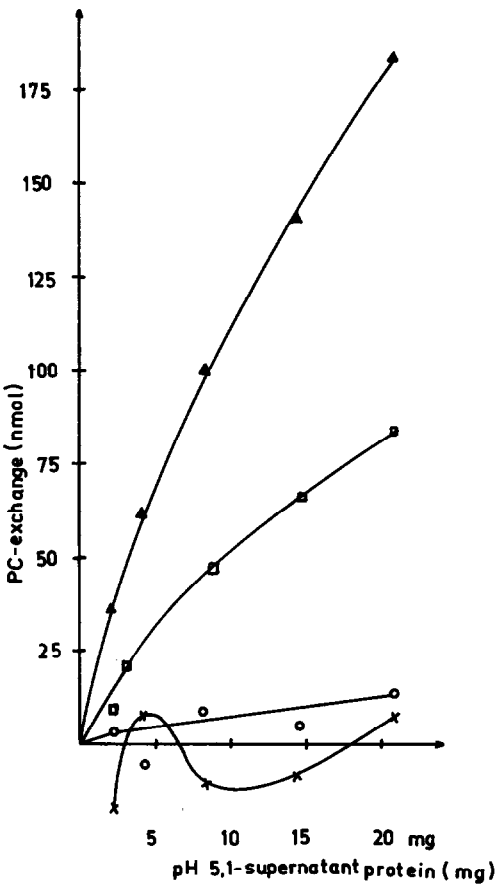

Fig.1. Exchange of PC molecular species substituted in positions 1 and 2 with the same fatty acid in relation to the PLEP (enriched in the pH 5.1-supernatant). The values are corrected for the passive exchange. Mitochondria 9.8 mg, liposomes 0.9 μmol of the following PC molecular species: (X——X) DPPC, (○——○) DSPC, (▲——▲) DOPC, (□——□) DLPC. Total vol. 4.5 ml, incubation was performed at 25°C for 60 min.

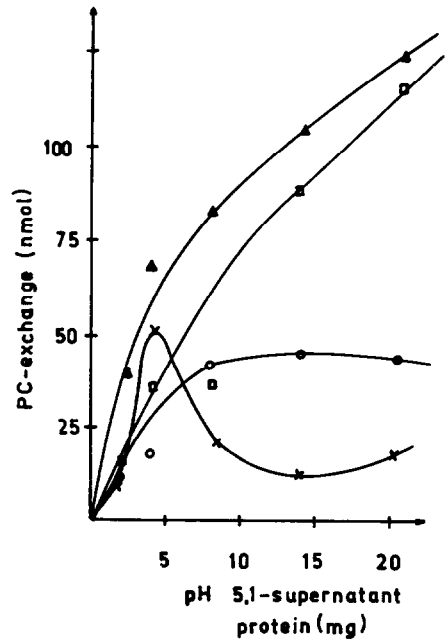

Fig.2. Exchange of PC substituted fatty acid specifically in position 2 only. Mitochondria 9 mg, liposomes 0.97 μmol of (X——X) PPC, (○——○) SPC, (▲——▲) OPC, (□——□) LPC. Incubation conditions as in fig.1.

the PC molecular species into two groups, containing saturated or unsaturated fatty acids in position 2. PC with unsaturated fatty acids show a many-fold greater exchange than PC with saturated ones. The passive values also vary with the PC molecular species but the relations are PPC:SPC:OPC:LPC 1:1:2:5 (32,28,68,150 nmol). Identical proportions were obtained for passive rates for PC disubstituted by the same fatty acids.

For the explanation of the results of active exchange some factors must be taken into consideration. The mechanism of PC exchange is a carrier process. Apart from the presence of PLEP, this can be influenced by its compartments: liposomal donor membranes and mitochondrial acceptors. Therefore, to extend the basic results in fig.1 and 2 two series of experiments were performed.

First the different physico–chemical properties of the PC liposomes and their influence on the protein-mediated exchange were investigated. Figure 3 shows that the exchanges of single PC molecular species remain different at 25°C, 37°C and 43°C. Certainly a

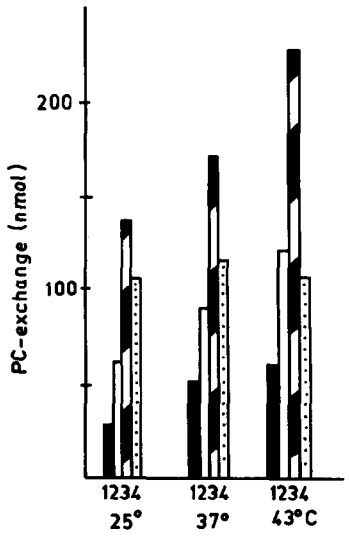

Fig.3. Exchange of PC molecular species at three temperatures: 25°C, 37°C and 43°C. 1=PPC, 2=SPC, 3=OPC, 4=LPC. Mitochondria 8 mg, pH 5.1-supernatant 18.5 mg protein. Incubation conditions as in fig.1.

different stimulation of the exchange with increasing temperature takes place with each single PC species. There is no correlation of the various stimulations with lipid phase transitions. The results presented in fig.3

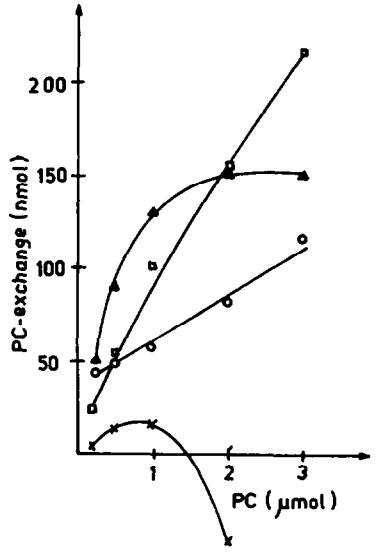

Fig.4. The exchange in relation to the PC concentration. Mitochondria 5 mg protein, pH 5.1-supernatant 22.9 mg protein. Reaction conditions as in fig.1. Symbols as in fig.2.

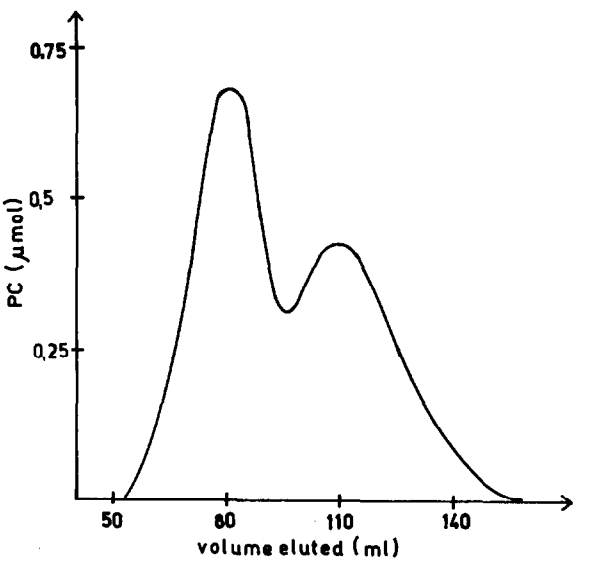

Fig.5. Elution diagram of 10 µmol PC (PPC and LPC respectively) on Sepharose 4B. Column height 40 cm, diameter 2.9 cm, speed of elution 20 ml/h, volume of the single fractions 5 ml.

exclude the possibility that the low exchange of PPC and DPPC is the consequence of different fluidities of the saturated PC molecular species in the liposomes. If this were so, a different exchange mechanism ought to exist at temperatures above and below the phase transition. PPC has a measured phase transition at 39°C, SPC at 57–59°C.

Another possibility is that the nature of liposomes influences the exchange. Different sizes and surfaces of the PC vesicles may exist due to different proportions of single to multilayer liposomes. The results of the fractionation of liposomes on Sepharose 4B are shown in fig.5. PC with linoleic or palmitic acid in position 2 have identical elution diagrams. We also investigated the exchange of OPC- and LPC-liposomes produced by sonication for 3 or 10 min respectively. The 3-min liposomes consist of a mixture of single and multilayered vesicles, the 10-min ones were only single walled. Vesicles different walls have equal influences on the exchange (table 1).

Differences of exchange were also demonstrated in relation to the PC concentration (fig.4). The LPC and SPC curves rise steadily, OPC attains a saturation limit near 1 µmol, PPC differs from other PC above concen-

Table 1
PC exchange with different types of liposomes

	(nmol)	Exchanged PC		
	3-min	Liposomes	10-min	Liposomes
	Passive	Active	Passive	Active
OPC	38	63	55	65
LPC	14.5	32	13.5	34

Mitochondria 8 mg protein, PC 0.63 μmol, pH 5.1-supernatant 23.5 mg protein. Reaction conditions as in fig.1.

trations of 1 μmol/incubation volume. The negative values are the result of a higher passive exchange probably due to a stronger adsorption or fusion of liposomes at mitochondria in the absence of PLEP.

In testing the influence of mitochondria on the different exchanges of PC molecular species we studied the protein-mediated (fig.6) and the passive exchanges of delipidated mitochondria. The passive values are very low but the relations between them are the same as those with whole mitochondria.

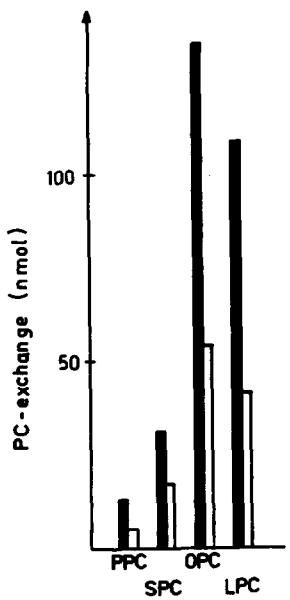

Fig.6. Exchange of 4 PC molecular species with delipidated mitochondria in relation to controls. Acceptor protein 8 mg, PC 1 μmol, pH 5.1-supernatant 25.3 mg protein. Black column = mitochondria, white column = delipidated mitochondria. Incubation conditions as in fig.1.

The experiments show 2 remarkable results:

(1) The active exchanges with the lipid-extracted mitochondria attain only half the values of the controls with whole mitochondria.

(2) The differences in the exchanges of PC molecular species remain constant.

It can be concluded that the selectivity is not necessarily related to the presence of PL in mitochondria. We therefore conclude, that the third component of the exchange process – the PLEP – must be the cause of the different exchanges. In further investigations we will try to purify the individual PLEPs.

Only a few authors have studied the exchange of PC molecular species. Taniguchi et al. [22] found no selectivity to the PC species. They performed their experiments at 4°C but it is known that the exchange is too low at this temperature to draw such a conclusion [1,7]. Another methodological objection must be made to the results of Parkes and Thompson [23] and Wirtz et al. [24]. The liposome–biological membrane assay system used by us has several advantages over exchange assays using two biological membranes. These advantages come from the fact that liposomes can be prepared from a single PC molecular species. This is not possible in the case of biological membranes. Besides, the PC species may be both metabolically heterogeneous and asymmetrically distributed.

Acknowledgement

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