Biochimica et Biophysica Acta, 773 (1984) 23–31
Elsevier

BBA 72068

TRANSFER OF PHOSPHATIDIC ACID BETWEEN MICROSOMAL AND MITOCHONDRIAL OUTER AND INNER MEMBRANES

JOLANTA BARAŃSKA and LECH WOJTCZAK

Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw (Poland)

(Received December 8th, 1983)

Key words: Phosphatidic acid; Phospholipid transfer protein; (Rat liver mitochondria)

(1) A protein fraction from rat liver cytoplasm, precipitable at 50–95% saturation of ammonium sulphate, binds phosphatidic acid from mitochondrial and microsomal membranes. Protein-bound phosphatidic acid was eluted from Sephadex G-75 in fractions corresponding to a molecular weight of about 10000. No such binding was observed with mitochondrial soluble proteins, either total or precipitated with ammonium sulphate between 50 and 95% saturation. (2) The transfer of phosphatidic acid from microsomes to mitochondria was increased by liver cytoplasmic proteins precipitable at 50–95% saturation of ammonium sulphate but not with mitochondrial soluble proteins. This increase by cytoplasmic proteins was pronounced in 200 mM sucrose but was negligible in 100 mM KCl where the spontaneous transfer was quite high. (3) Cytoplasmic proteins stimulated the synthesis of cardiolipin and phosphatidylglycerol in mitochondria deprived of the outer membrane but not in intact mitochondria when phosphatidic acid was supplied either by microsomes or liposomes. (4) It is suggested that the transfer of phosphatidic acid from the outer to the inner mitochondrial membrane is not mediated by transfer proteins but occurs either by direct contact of the membranes or as free diffusion through the aqueous phase.

Introduction

Mitochondria are lacking in enzyme systems for a complete synthesis of complex phospholipids (cf. Ref. 1). Their synthetic ability is limited to phosphatidic acid and to most characteristic mitochondrial phospholipids, viz. phosphatidylglycerol and cardiolipin. The latter two phospholipids are synthesized in the inner mitochondrial membrane [2,3], whereas the synthesis of phosphatidic acid occurs in the outer mitochondrial membrane and the endoplasmic reticulum [4–7]. Thus, in order to be used for a subsequent synthetic process, phosphatidic acid has to be transferred from the outer to the inner mitochondrial membrane. Phospholipid transfer proteins that catalyze the intracellular exchange of phospholipids have been found in the cytoplasm of several tissues [8,9]. Preliminary results from this laboratory [10] have shown that rat liver cytoplasm accelerates the transfer of phosphatidic acid from liposomes to mitochondria. More recently, Crain and Zilversmit [11] isolated from bovine liver cytoplasm two low-molecular-weight proteins that are capable of transferring this phospholipid between membranes. A similar ability of guinea-pig liver cytoplasmic proteins was demonstrated by Stuhne-Sekalec and Stanacev [12].

The present investigation was aimed to look for phosphatidic acid transfer proteins in the intermembrane compartment of liver mitochondria. The results confirmed the presence of protein(s) which binds and transports phosphatidic acid in the cytoplasmic fraction, but we were unable to show the same for mitochondrial soluble proteins.

Materials and Methods

Biological material

Liver mitochondria, microsomes and the soluble cytoplasmic fraction from Wistar rats were isolated by conventional procedures [13]. Mitochondria depleted of the outer membrane (mitoplasts) were prepared according to Schnaitman and Greenawalt [14]. Soluble mitochondrial proteins were obtained by sonifying the mitochondria (5 × 30 s) and removing membranes by centrifugation at 100 000 × *g* for 60 min. A partial purification of soluble proteins from both the cytoplasm and mitochondria was achieved essentially as described by Bloj and Zilversmit [15]. In short, the 100 000 × *g* supernatant was adjusted to pH 5.1 and precipitated proteins and lipoproteins were discarded. The supernatant was then adjusted to pH 7.4 and ammonium sulphate was added to 50% saturation. The precipitate was discarded and the solution was made 95% saturated with ammonium sulphate. Proteins that precipitated after 2 h standing at 4°C were collected, dissolved in 10 mM Tris-HCl (pH 7.4)/5 mM 2-mercaptoethanol/3 mM NaN_3 and dialyzed against the same solution during 24–48 h at 4°C. At this point mitochondrial and cytoplasmic proteins were used for some experiments. For other experiments the proteins were further fractionated by filtration through two columns of Sephadex G-75 (80 × 5 cm) equilibrated with 10 mM Tris-HCl/5 mM 2-mercaptoethanol/3 mM NaN_3 (pH 7.4) as described previously [16]. The flow rate was 40 ml/h and 10 ml fractions were collected and monitored for protein at 280 nm. Fractions 24 to 39 and 40 to 50 corresponded to the high- and low-molecular-weight proteins, respectively. The material was freshly prepared for each experiment.

Mitochondria and microsomes loaded with [^{32}P]phosphatidic acid were prepared as follows [5]. The particles (100–160 mg protein) were incubated in 10 ml of the medium containing 60 mM KCl, 8 mM Tris-HCl (pH 7.4), 7 mM ATP, 6 mM $MgCl_2$, 2 mM NaN_3, 0.3 mM sodium palmitate, 0.2 mM CoASH and 0.6 mM glycerol 3-[^{32}P]phosphate (10^8 cpm). After 1 h at 30°C the particles were sedimented by centrifugation and washed several times with 250 mM sucrose/1 mM EDTA/1 mM glycerol 3-phosphate. For some binding experiments, the membranes were heat-coagulated for 3 min at 100°C. For transfer experiments microsomes were sonicated 4 × 30 s, and non-dispersed clumps removed by centrifugation at 10 000 × *g* for 10 min.

Incubations

This was carried out under constant shaking at 30°C or 37°C with air as the gas phase.

The binding of phosphatidic acid was examined by incubating microsomes or mitochondria containing [^{32}P]phosphatidic acid with cytoplasmic or mitochondrial proteins obtained by ammonium sulphate precipitation or with the cytoplasmic high- or low-molecular-weight protein fractions obtained by Sephadex filtration. After incubation, the particles were removed by centrifugation, the supernatants were filtered through two Sephadex G-75 columns as described in the preceding section and the radioactivity was measured in the effluent.

The transfer of phosphatidic acid was measured using labelled microsomes as the donor and mitochondria as the acceptor. At various periods of time 0.25 ml aliquots of the incubation mixture were transferred into centrifugation tubes filled with 5 ml cold 250 mM sucrose/1 mM EDTA, and rapidly centrifuged at 10 000 × *g*; the resulting mitochondrial pellet was washed with the same solution. The final pellet was dissolved in formic acid and counted for radioactivity.

The synthesis of mitochondrial phospholipids was examined by incubating mitochondria (5–6 mg protein/ml) at 30°C in the medium containing 10 mM Tris-HCl (pH 7.4)/7 mM ATP/5 mM 2-mercaptoethanol/3 mM $MgCl_2$/3 mM NaN_3/2 mM CTP/0.5 mM EGTA/0.4 mM CoASH/0.3 mM sodium palmitate/1 mM glycerol 3-[^{32}P]phosphate corresponding to about 100 000 cpm per ml and either 100 mM KCl or 200 mM sucrose. After 1, 2 and 3 h, aliquots of the mixture were rapidly centrifuged, the mitochondrial pellet was extracted with chloroform and methanol and the phospholipids were separed by thin-layer chromatography. The spots were visualized by autoradiography, scraped and counted for radioactivity.

Lipid extraction and chromatography

Mitochondrial lipids were extracted using

methanol/chloroform (1 : 2, v/v) [17] or, in experiments in which estimation of lysophospholipids was essential, butanol [18]. Phospholipids were separated and identified by thin-layer chromatography on silica gels G and H (Merck AG, Darmstadt, F.R.G.) developed with $CHCl_3/CH_3OH/28\%\ NH_4OH/H_2O$ (65 : 35 : 2.5 : 2.5, v/v) [19] or with $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (65 : 25 : 2 : 4, v/v) [18]. Spots were visualized using iodine vapour and autoradiography.

Liposome preparation

Liposomes made of total mitochondrial lipids containing [^{32}P]phosphatidic acid were prepared essentially as described previously [20]. Mitochondrial lipids after extraction were washed with 0.9% NaCl, dispersed in 250 mM sucrose/2 mM Tris-HCl (pH 7.4)/1 mM EDTA and sonicated in a 60 W sonicator (MSE, London, U.K.) at maximum output during 20 min under air and cooling in ice bath. The liposomes were used immediately after preparation.

Enzyme assay

NADPH-cytochrome *c* reductase (EC 1.6.2.4) was measured as described by Sottocasa et al. [21].

Chemicals and other procedures

rac-Glycerol 3-[^{32}P]phosphate was prepared by heating inorganic [^{32}P]phosphate obtained from the Institute of Nuclear Research (Świerk, Poland) with glycerol [22].

Protein was determined by the biuret method [23]. In the effluent from the Sephadex column, protein content was monitored by measuring light absorption at 280 nm.

Radioactivity was measured in a scintillation spectrometer making use of the Čerenkov effect for ^{32}P [24].

Results

The ability of rat liver cytoplasmic proteins to bind phosphatidic acid is shown in Fig. 1. In this experiment cytoplasmic proteins obtained by precipitation with ammonium sulphate were in-

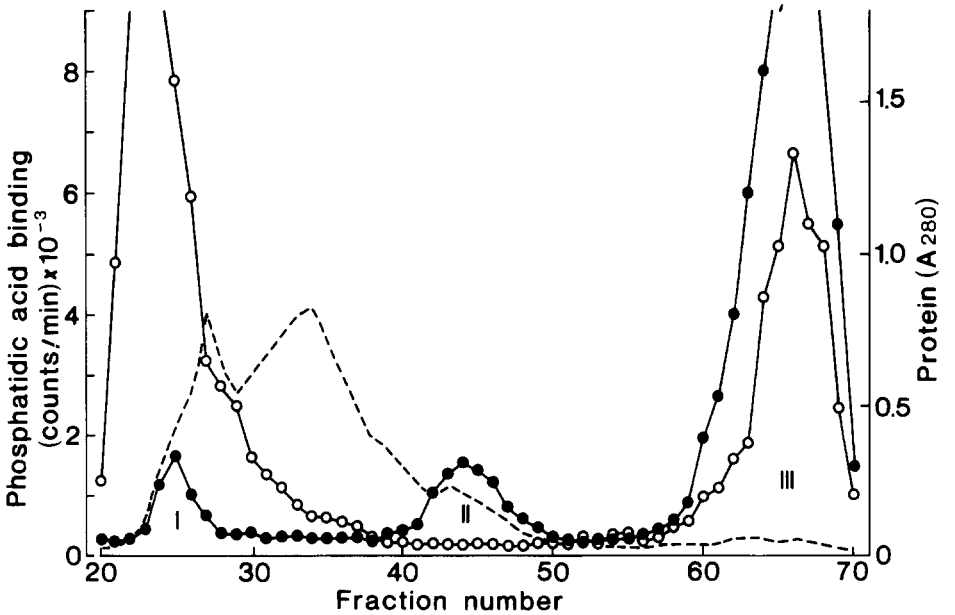

Fig. 1. Elution profile of cytoplasmic proteins loaded with [^{32}P]phosphatidic acid. Heat-coagulated mitochondria (150 mg protein, 500000 cpm [^{32}P]phosphatidic acid) were incubated with cytoplasmic proteins (150 mg) obtained by ammonium sulphate precipitation in 250 mM sucrose/10 mM Tris-HCl (pH 7.4)/5 mM 2-mercaptoethanol/3 mM NaN_3 in final volume of 10 ml at 37 °C for 1 h. In the control, cytoplasmic proteins were omitted. After sedimenting the particles, the clear supernatant was passed through Sephadex G-75 as described under Materials and Methods. ●———●, Radioactivity in fractions from the sample incubated with cytoplasmic proteins; — — —, absorption at 280 nm in the same fractions; ○———○, radioactivity in fractions from the control.

cubated with heat-coagulated mitochondria which had previously accumulated [^{32}P]phoshatidic acid. After sedimenting the particles by high-speed centrifugation the supernatant was passed through Sephadex G-75 columns. In the effluent three peaks of radioactivity were resolved. Peak I corresponded to the void volume of the column. The form of phosphatidic acid it contained has not been elucidated. It might be present there as finely dispersed membrane fragments and/or phospholipid micelles. It could not be excluded that it also contained phosphatidic acid tightly bound to a high molecular weight protein. The latter assumption is, however, rather unlikely, since peak I also appeared in the control run, i.e., when the particles were incubated without the cytoplasmic fraction. In fact, in this case peak I was much higher than after incubation with cytoplasmic proteins (Fig. 1). This suggests that phosphatidic acid of peak I was degraded, presumably by phosphatidate phosphohydrolase (EC 3.1.3.4) present in our ammonium sulphate fraction of cytoplasmic proteins. This is confirmed by the observations that (1) peak III containing small molecules increased when peak I decreased and (2) peak I was not diminished if the low molecular weight protein fraction was used instead of the whole ammonium sulphate precipitate (not shown). Peak III, containing mostly inorganic [^{32}P]phosphate and some glycerol [^{32}P]phosphate, was also present in the control runs because these contaminants were always present in our phosphatidic acid-loaded particles.

In contrast to peaks I and III, peak II was recovered only in the presence of cytoplasmic proteins (Fig. 1). It contained compounds of molecular mass of the order of 10^4 (as calibrated with cytochrome *c*) and corresponded to phosphatidic acid bound to low molecular weight protein(s). The same peak of radioactivity was also observed when the ammonium sulphate precipitate was replaced by the low molecular weight protein fraction obtained by Sephadex filtration and was absent when the high molecular weight protein fraction was used. An identical picture was obtained when microsomes loaded with [^{32}P]phosphatidic acid were used as donors. Practically the total label present in peak II was in phosphatidic acid with negligible amounts of lysophosphatidic acid only.

The problem of whether mitochondrial soluble proteins, similar to the case with cytoplasmic proteins, can bind phosphatidic acid was examined in two types of experiment. In the first one, mitochondria from four rats were incubated with glycerol 3-[^{32}P]phosphate and cofactors required for the synthesis of phosphatidic acid (see Materials and Methods). After incubation, mitochondria were centrifuged, washed and sonicated. The membranes were sedimented by high-speed centrifugation and the resulting supernatant containing intermembrane and matrix proteins was applied on a Sephadex G-75 column. In the effluent only two peaks of radioactivity were observed, corresponding to peaks I and III obtained by gel filtration of cytoplasmic proteins. In the second type of experiment, mitochondrial proteins precipitated with ammonium sulphate between 50% and 95% saturation were used. They were incubated with heat-coagulated mitochondria that had accumulated [^{32}P]phosphatidic acid. After gel filtration, again only two peaks of radioactivity were recovered (Fig. 2).

These results cannot exclude a possibility that mitochondrial proteins capable to bind phosphatidic acid are present in the void volume peak as aggregates with phospholipid micelles. Therefore, further experiments were performed with the aim of examining the ability of soluble proteins to transfer phosphatidic acid. This was investigated in a system in which microsomes loaded with [^{32}P]phosphatidic acid were incubated with mitochondria. To minimize obscuring of the results by accumulation in mitochondria of inorganic [^{32}P]phosphate (resulting from hydrolysis of [^{32}P]phosphatidic acid) the medium was supplemented with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (to collapse the transmembrane potential and pH gradient) and inorganic phosphate. It was found that in the sucrose medium cytoplasmic proteins stimulated the rate of phosphatidic acid transfer, whereas mitochondrial soluble proteins were without effect (Fig. 3B). In KCl medium, the spontaneous transfer of phosphatidic acid was much higher and the stimulation by cytoplasmic proteins less accentuated or even not visible, as seen in Fig. 3A, and again mitochondrial proteins had no effect. Similar negative results were obtained if ammonium sulphate precipitate

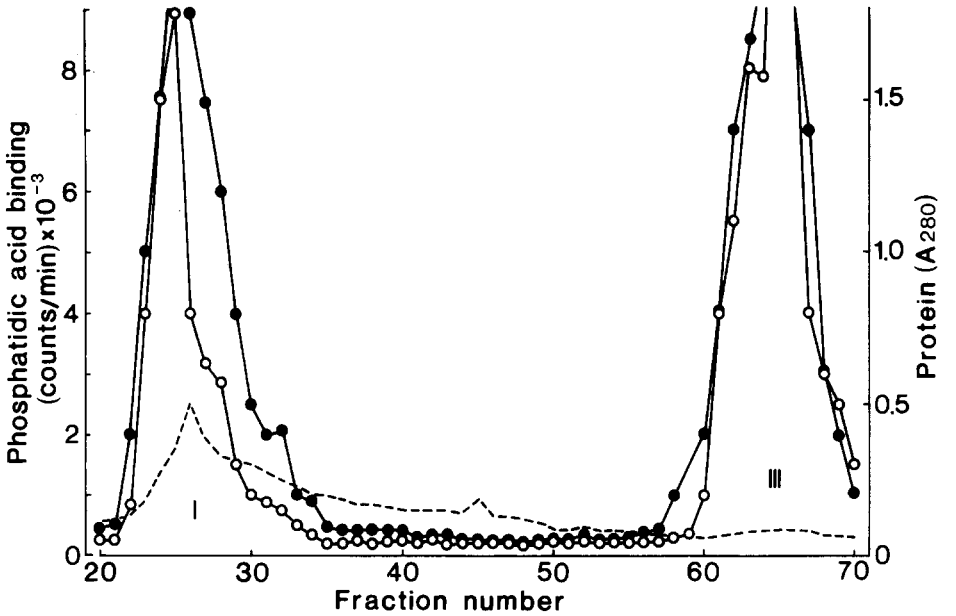

Fig. 2. Elution profile of mitochondrial soluble proteins incubated with mitochondria containing [^{32}P]phosphatidic acid. Heat-coagulated mitochondria (185 mg protein, 600000 cpm [^{32}P]phosphatidic acid) were incubated with mitochondrial soluble proteins (60 mg) obtained by ammonium sulphate precipitation in the medium and under conditions as in Fig. 1. ●———●, Radioactivity in fractions from the sample incubated with mitochondrial soluble proteins: — — —, absorption at 280 nm in these fractions; ○———○, radioactivity in fractions from the control.

of soluble mitochondrial proteins, instead of total soluble proteins, was used. As shown in the legend to Fig. 3, a partial hydrolysis of phosphatidic acid occurred during 1 h incubation. This was stimulated by the cytoplasmic fraction in the KCl medium, in accordance with the observation [36] that phosphatidate phosphohydrolase is activated by 0.1 M monovalent cations.

The time-dependent transfer of phosphatidic acid in the KCl medium (Fig. 3A) and with cytoplasmic proteins in the sucrose medium (Fig. 3B) could not be accounted for by an increasing degree of microsomes co-sedimenting with mitochondria. This was checked in a separate experiment by measuring NADPH-cytochrome *c* reductase, a microsomal marker, in the mitochondrial pellet obtained after incubation with microsomes. It was found that this co-sedimentation did not increase with time in any of the incubation systems.

The question as to whether cytoplasmic proteins can mediate the transfer of phosphatidic acid from the outer to the inner membrane of intact mitochondria was examined in two types of experiment. In the first one, mitochondria were incubated with glycerol 3-[^{32}P]phosphate and cofactors for the synthesis of phosphatidic acid and cardiolipin (see Materials and Methods). After reisolation of mitochondria, lipids were extracted and the incorporation of ^{32}P into various phospholipids was examined. It was found that most of lipid ^{32}P was recovered in phosphatidic acid. Only after 3 h incubation was any substantial incorporation into phosphatidylglycerol and cardiolipin observed, amounting to 4% and 3%, respectively. Addition of the cytoplasmic proteins (ammonium sulphate precipitate) or the low-molecular-weight fraction therefrom somewhat decreased the amount of phospholipid ^{32}P recovered in mitochondria (because of binding of phosphatidic acid that was formed), but had no effect on the percentage distribution of ^{32}P among phosphatidic acid, phosphatidylglycerol and cardiolipin. This indicates that cytoplasmic proteins that bind and transfer phosphatidic acid did not enhance the synthesis of phosphatidylglycerol and cardiolipin in the inner membrane.

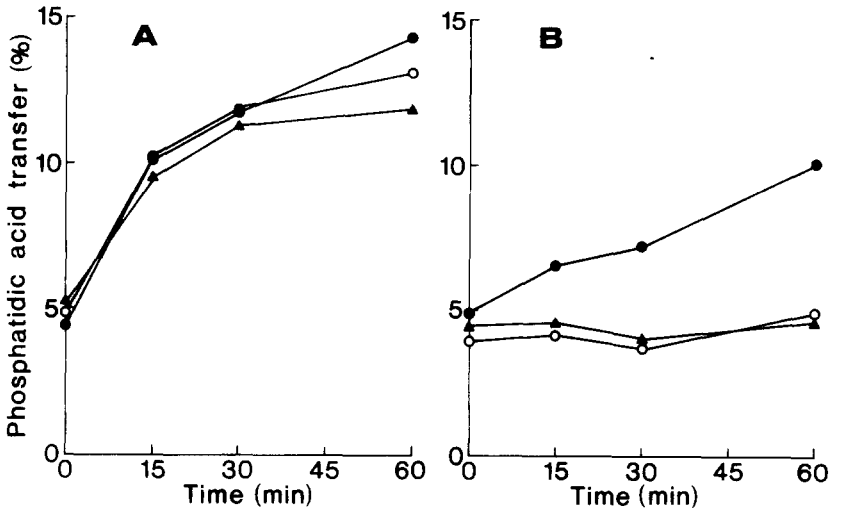

Fig. 3. Transfer of phosphatidic acid from microsomes to mitochondria. Mitochondria (6.4 mg protein/ml) were incubated with microsomes (6 mg protein/ml) containig [^{32}P]phosphatidic acid (400000 cpm per ml), at 37°C in the medium containing either 100 mM KCl (A) or 200 mM sucrose (B) and 10 mM Tris-HCl (pH 7.4), 5 mM 2-mercaptoethanol, 3 mM NaN_3, 2 mM EDTA, 2 mM phosphate, 2 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and the following additions: ○, none (control); ●, cytoplasmic proteins (ammonium sulphate precipitate), 12.5 mg/ml; ▲, soluble mitochondrial proteins, 12.0 mg/ml. The transfer was measured as described under Materials and Methods. After 60 min incubation the following percentage of the total radioactivity was found in the water-soluble, butanol-insoluble [18], fraction: in KCl medium: control, 22%; with cytoplasmic proteins, 30%; with mitochondrial proteins, 18%; in sucrose medium: all samples, 16%. This fraction corresponded mostly to inorganic phosphate.

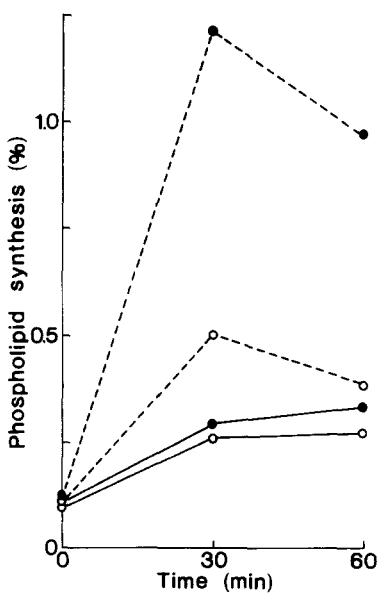

Fig. 4. Synthesis of mitochondrial phospholipids from exogenous phosphatidic acid. Mitochondria (8 mg protein/ml) or the equivalent amount of mitoplasts were incubated at 30°C with microsomes (8 mg protein/ml) containing [^{32}P]phosphatidic acid (200000 cpm per ml) in the medium containing 200 mM sucrose, 10 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 3 mM NaN_3, 2 mM CTP, 2 mM EGTA, 1 mM *rac*-glycerol 3-phosphate and cytoplasmic proteins (ammonium sulphate precipitate, 14.5 mg/ml) were indicated. 1 ml aliquots were rapidly centrifuged, the mitochondrial or mitopast pellet was extracted and lipids were separated by thin-layer chromatography and counted for radioactivity. Each point represents the sum of the radioactivity, expressed as percentage of the total radioactivity added as phosphatidic acid, in spots corresponding to cardiolipin, phosphatidylglycerol and CDPdiacylglycerol, and is a mean value of the parallel chromatographic runs. ———, mitochondria; ------, mitoplasts; ●, with cytoplasmic proteins; ○, without cytoplasmic proteins.

In another type of experiment, mitochondria were incubated with microsomes containing [^{32}P]phosphatidic acid in the presence of glycerol 3-phosphate and CTP that are required for the synthesis of phosphatidylglycerol and cardiolipin from phosphatidic acid. As shown in Fig. 4, cytoplasmic proteins were without effect on the rate of ^{32}P incorporation into these phospholipids in intact mitochondria. In contrast, cytoplasmic proteins greatly increased the synthesis of phosphatidylglycerol and cardiolipin in mitoplasts. The latter observation, as well as the fact that the incorpora-

tion in mitoplasts was higher than in intact mitochondria, strongly suggests that the transfer of phosphatidic acid between the outer and the inner membrane was the limiting factor in the synthesis of phosphatidylglycerol and cardiolipin under experimental conditions.

An argument that the lower rate of [^{32}P]phosphatidic acid incorporation into phosphatidylglycerol and cardiolipin in intact mitochondria is due to a dilution by unlabelled phosphatidic acid present in the outer membrane is very unlikely, because phosphatidic acid is virtually absent from mitochondria unless they are preincubated under conditions promoting its synthesis [5]. This was not the case in the experiments described here, in which ATP was not added and its synthesis was prevented by sodium azide [25].

A similar experiment with liposomes containing [^{32}P]phosphatidic acid as donor membranes was also carried out. Also in this case, cytoplasmic proteins did not increase the transfer of phosphatidic acid into the inner membrane, since the sum of the incorporation into phosphatidylglycerol, cardiolipin and CDPdiacylglycerol was the same without and with cytoplasmic proteins (2.25% and 2.00%, respectively).

Discussion

The presence in liver cytoplasm of transfer protein(s) for phosphatidic acid has been documented so far by independent reports from two laboratories [11,12]. The present investigation provides a further evidence for the occurrence of such protein(s) in the cytoplasm of rat liver. By means of gel filtration on Sephadex G-75 we showed that the ability to bind phosphatidic acid was confined to protein(s) of molecular weight around 10000. A similar elution pattern has been previously observed for the binding and transferring activity for phosphatidylserine [16]. Although the ability to bind phosphatidic acid is not a sufficient proof for the transferring property, it provides an indication in this direction. It may thus be supposed that phosphatidic acid is transported by the low-molecular-weight, unspecific, transfer protein identified in rat liver by Bloj and Zilversmit [15]. A broad phospholipid spectrum and the molecular weight in the range of 13000–14500 are also characteristic for the phosphatidic acid-transferring protein from bovine liver [11].

In a preliminary communication from this laboratory [10], we have reported that various proteins can enhance the transfer of phosphatidic acid between membranes. However, in the present investigation we have been able to confirm this only for the low-molecular-weight protein fraction from liver cytoplasm. In particular, we were unable to demonstrate either the binding or the transfer of phosphatidic acid by soluble proteins from liver mitochondria.

To explain the mechanism of the transfer of phosphatidic acid between the outer and the inner mitochondrial membrane one might speculate that the outer membrane is permeable to low-molecular-weight phospholipid transfer proteins that are present in the liver cell and have been identified in the cytoplasm [15,16,26]. If so, one should expect that isolated mitochondria are deficient in these proteins, as they have leaked out during the isolation procedure. This is not very likely in view of the known impermeability of the outer membrane to compounds of molecular weight above 5000 [27]. Nevertheless, such a possibility could not be excluded a priori. It was found, however, that the synthesis of phosphatidylglycerol and cardiolipin in the inner membrane was not increased by the cytoplasmic protein fraction when phosphatidic acid was either synthesized in the outer membrane from its precursors or provided from externally added microsomes (Fig. 4) or liposomes. This shows that the externally added transfer proteins are not present or, at least, not functioning in the intermembrane compartment. In constrast, the transfer protein(s) increased the synthesis of inner membrane phospholipids from externally added phosphatidic acid in mitoplasts, i.e., under conditions when the protein(s) had an immediate access to the inner membrane.

In view of these negative findings two possibilities for the transfer of phosphatidic acid within the mitochondrial intermembrane compartment can be considered. First, that this compartment does indeed contain a transfer protein for phosphatidic acid which is, however, present in such small quantity that it could not be detected in mitochondrial extracts by methods applied in this study. The intermembrane compartment accounts

only for a minute fraction of the total mitochondrial volume and therefore even a small amount of the transfer protein migth result in a concentration high enough to attain a high rate of phosphatidic acid transfer between membranes of the intact mitochondrion. An alternative possibility is the transfer of phosphatidic acid between the outer and the inner membrane that is not mediated by transfer proteins.

Although the first possibility cannot be excluded, we believe that the second one is highly likely. Stuhne-Sekalec and Stancev [12] have already demonstrated a high 'spontaneous' transfer of phosphatidic acid between membranes. In the present investigation we have also shown that in the absence of the cytoplasmic proteins phosphatidic acid was transferred from microsomes to mitochondria at a relatively high rate. In fact, at high ionic strength, the 'spontaneous' transfer was so high that it was scarcely stimulated by cytoplasmic proteins (Fig. 3).

A large body of evidence has accumulated that phospholipids can be transferred between membranes without the participation of transfer proteins [28], and the mechanism of this process has been mainly suggested as free diffusion of phospholipids through the aqueous phase in micellar and/or monomeric form [29–32]. It may be expected that phosphatidic acid is especially suited to form micelles because of its highly negative polar head group. In fact, in the present investigation the portion of phosphatidic acid that could be extracted from the membranes by simple washing with sucrose probably represented the formation of micelles, as it was eluted in the void volume of the Sephadex column (Figs. 1 and 2).

A transfer of phospholipids between the outer and the inner membrane of mitochondria, independent of transfer proteins, has already been suggested by Blok et al. [33]. These authors proposed that such mechanism was enabled by a contaguous structure of the mitochondrial membranes. In fact, the distance between the two mitochondrial membranes in situ ('orthodox' configuration) is extremely small. Moreover, the ionic strength of the cytoplasm resembles that of the ionic medium used in the present investigation at which the protein-independent phosphatidic acid transfer was quite high.

In conclusion, we propose that phosphatidic acid is transported from the outer mitochondrial membrane to the inner one most likely either by a direct contact of the two membranes or by diffusion through the water phase in micellar and/or monomeric forms. This transfer is certainly facilitated by the fact that phosphatidic acid is synthesized on the internal side of the outer membrane [34,35]. In view of the present results is also seems likely that the transfer of phosphatidic acid within the cytoplasmic compartment of the cell can proceed to some extent in a similar way.

References

1 McMurray, W.C. (1973) in Form and Function of Phospholipids (Ansel, G.B., Hawthorne, J.N. and Dawson, R.M.C., eds.), BBA Library, Vol. 3, pp. 205–251, Elsevier, Amsterdam
2 Hostetler, K.Y. and Van den Bosch, H. (1972) Biochim. Biophys. Acta 260, 380–386
3 Stanacev, N.Z., Davidson, J.B., Stuhne-Sekalec, L. and Domazet, Z. (1973) Can. J. Biochem. 51, 286–304
4 Stoffel, W. and Schiefer, H.G. (1968) Z. Physiol. Chem. 349, 1017–1026
5 Zborowski, J. and Wojtczak, L. (1969) Biochim. Biophys. Acta 187, 73–84
6 Daae, L.N.W. and Bremer, J. (1970) Biochim. Biophys. Acta 210, 92–104
7 Bremer, J., Bjerve, K.S., Borrebaek, B. and Christiansen, R. (1976) Mol. Cell. Biochem. 12, 113–125
8 Wirtz, K.W.A. (1974) Biochim. Biophys. Acta 344, 95–117
9 Zilversmit, D.B. and Hughes, M.E. (1976) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 7, pp. 211–259, Plenum Press, New York
10 Wojtczak, L. and Zborowski, J. (1975) 10th Meeting Federation of European Biochemical Societies, Paris, Abstracts, p. 1042
11 Crain, R.C. and Zilversmit, D.B. (1980) Biochemistry 19, 1433–1439
12 Stuhne-Sekalec, L. and Stanacev, N.Z. (1980) Can. J. Biochem. 58, 1082–1090
13 Hogeboom, G.H. (1955) Methods Enzymol. 1, 16–19
14 Schnaitman, C. and Greenawalt, J.W. (1969) J. Cell Biol. 38, 158–175
15 Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613–1619
16 Barańska, J. and Grabarek, Z. (1979) FEBS Lett. 104, 253–257
17 Wojtczak, L., Włodawer, P. and Zborowski, J. (1963) Biochim. Biophys. Acta 70, 290–305
18 Bjerve, K.S., Daae, L.N.W. and Bremer, J. (1974) Anal. Biochem. 58, 238–245
19 Barańska, J. (1980) Biochim. Biophys. Acta 619, 258–266
20 Barańska, J. and Wojtczak, L. (1976) FEBS Lett. 71, 83–86

21 Sottocasa, G.L., Kuylenstierna, B., Ernster, E. and Bergstrand, A. (1967) J. Cell Biol. 32, 415–438
22 Kennedy, E.P. (1953) J. Biol. Chem. 201, 399–412
23 Gornall, A.G., Bardawill, C.J. and David, M. (1949) J. Biol. Chem. 177, 751–766
24 Gould, J.M., Carther, R. and Winget, G.D. (1972) Anal. Biochem. 50, 540–548
25 Bogucka, K. and Wojtczak, L. (1966) Biochim. Biophys. Acta 122, 381–392
26 Zilversmit, D.B. and Hughes, M.E. (1977) Biochim. Biophys. Acta 469, 99–110
27 Pfaff, E., Klingenberg, M., Ritt, E. and Vogell, W. (1968) Eur. J. Biochem. 5, 222–232
28 Smith, L.C. and Scow, R.O. (1979) Prog. Biochem. Pharmacol. 15, 109–138
29 Martin, F.J. and MacDonald, R.C. (1976) Biochemistry 15, 321–327
30 Papahadjopoulos, D., Hui, S., Vail, W.J. and Poste, G. (1976) Biochim. Biophys. Acta 448, 245–264
31 Roseman, M.A. and Thompson, T.E. (1980) Biochemistry 19, 439–444
32 McLean, L.R. and Phillips, M.C. (1981) Biochemistry 20, 2893–2900
33 Blok, M.C., Wirtz, K.W.A. and Scherphof, G.L. (1971) Biochim. Biophys. Acta 233, 61–75
34 Nimmo, H.G. (1979) FEBS Lett, 101, 262–264
35 Carroll, M.A., Morris, P.E., Grosjean, C.D., Anzalone, T. and Haldar, D. (1982) Arch. Biochem. Biophys. 214, 17–25
36 Sedgwick, B. and Hübscher, G. (1967) Biochim. Biophys. Acta 144, 397–408