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Characterization of a microsomal subfraction associated with mitochondria of the yeast, *Saccharomyces cerevisiae*. Involvement in synthesis and import of phospholipids into mitochondria

Barbara Gaigg, Renate Simbeni, Claudia Hrastnik, Fritz Paltauf, Günther Daum *

Institut für Biochemie und Lebensmittelchemie, Technische Universität Graz, Petersgasse 12 / 2, A-8010 Graz, Austria

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Abstract

In the yeast, *Saccharomyces cerevisiae*, similar to higher eukaryotes most phospholipids are synthesized in microsomes. Mitochondria contribute to the cellular biosynthesis of phospholipids insofar as they harbor phosphatidylethanolamine decarboxylase, and enzymes of phosphatidylglycerol and cardiolipin synthesis. In this paper we present evidence that certain enzymes of phospholipid biosynthesis, namely phosphatidylserine and phosphatidylinositol synthase, are enriched in a special *m*icrosomal fraction *a*ssociated with *m*itochondria, which we named MAM. This fraction was isolated and characterized with respect to marker enzymes, protein and phospholipid composition, and enzymes of phospholipid synthesis. According to these analyses MAMs are a specialized subfraction of the endoplasmic reticulum, which is distinct from other microsomal subfractions. Phosphatidylserine synthesized in MAMs can be readily imported into mitochondria and converted to phosphatidylethanolamine. Reassociation of MAMs with purified mitochondria led to reconstitution of the import of phosphatidylserine into mitochondria. Organelle contact is suggested as a possible mechanism of this process.

Keywords: Phosphatidylserine; Phosphatidylethanolamine; Mitochondrion; Microsome; (Yeast); (*S. cerevisiae*)

1. Introduction

Phospholipid synthesis in mitochondria of the yeast, *Saccharomyces cerevisiae*, as well as of higher eukaryotes is restricted to the formation of phosphatidylethanolamine (by decarboxylation of phosphatidylserine), phosphatidylglycerol and cardiolipin [1,2]. However, mitochondrial membranes, like all other cellular membranes, contain the whole set of cellular phospholipids, among them phosphatidylcholine, phosphatidylinositol and phosphatidylserine, which are not synthesized within the mitochondrion. This fact necessitates an efficient translocation of these phospholipids from their site of synthesis into mitochondria. Import of phosphatidylserine is of special interest, because it serves as the substrate for the biosynthesis of phosphatidylethanolamine catalyzed by phosphatidylserine decarboxylase, which is located on the outer aspect of the inner mitochondrial membrane [3]. In order to reach its site of metabolic conversion extramitochondrially synthesized phosphatidylserine has to traverse the outer mitochondrial membrane. The metabolic conversion of phosphatidylserine to phosphatidylethanolamine within the mitochondrion provides a convenient system to measure the import of phosphatidylserine into this organelle. This assay had been used previously to study phosphatidylserine traffic in mammalian cells [4–7] and in yeast [8].

The question as to the subcellular site of synthesis of those phospholipids that are destined for import into mitochondria is still unsolved. Former work from our laboratory [2] had revealed that a specific subfraction of microsomes contained the highest specific activity of various phospholipid-synthesizing enzymes. Vance [9] reported about a membrane fraction associated with mitochondria which exhibits high specific activities of several enzymes of phospholipid biosynthesis. An involvement of this fraction in the translocation of phospholipids between mammalian microsomes and mitochondria was suggested. More recently Ardail et al. [10] demonstrated an interaction of contact sites between the outer and the inner mitochondrial membrane with a specialized microsomal fraction thus forming a complex that catalyzes concerted synthesis of

* Corresponding author. Fax: +43 316 810599.

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phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine.

In the present paper we demonstrate that similar to mammalian cells a special subfraction of microsomes is associated with yeast mitochondria. In addition to the biochemical characterization of this compartment we present evidence that phosphatidylserine synthesized in this fraction is readily imported into mitochondria in vitro, and phospholipid flow via membrane contact is the most likely mechanism for this translocation event.

2. Materials and methods

2.1. Strains and culture conditions

The wild-type yeast strain *Saccharomyces cerevisiae* D273-10B and a revertant strain of the phosphatidylserine synthase deficient mutant *Saccharomyces cerevisiae cho1* SDK03-1A [11], kindly provided by S.D. Kohlwein, were used throughout these studies. Both strains were cultivated under aerobic conditions at 30° C. Wild-type cells were grown on a medium containing 2% lactate as a carbon source as described before [12]. The revertant strain of the *cho1* mutant SDK03-1A was grown on YPD-medium containing 3% glucose, 1% yeast extract (Oxoid), and 2% peptone (Oxoid) under aerobic conditions at 30° C. Cells were always harvested in the logarithmic phase.

2.2. Isolation of purified mitochondria, MAMs (microsomes associated with mitochondria), and other microsomal subfractions

Yeast spheroplasts and 'crude' mitochondria were isolated by published procedures [12]. Microsomal fractions were obtained from the postmitochondrial supernatant by successive steps of differential centrifugation at 30 000, 40 000 and 100 000 × *g*. The respective membrane pellets were washed twice with 0.6 M mannitol, 10 mM Tris-HCl, pH 7.4. Isolation of microsomes associated with mitochondria (MAMs) is based on treatment of 'crude' mitochondrial preparations with buffer at pH 6.0. Mitochondria were suspended in buffer A (0.6 M sorbitol, 5 mM Mes, pH 6.0) and layered on top of a continuous sucrose gradient (20–50% sucrose in buffer A). After 1 h of centrifugation at 100 000 × *g* 'purified' mitochondria were harvested from the lower third of the tube, diluted 3-fold with buffer A and centrifuged for 10 min at 12 000 × *g*. The resulting pellet was washed once with buffer B (0.6 M mannitol, 10 mM Tris-HCl, pH 7.4) and resuspended in a small volume of buffer B. The MAM fraction was removed from the top of the gradient, diluted 3-fold with buffer A and centrifuged for 1 h at 100 000 × *g*. The resulting pellet was resuspended in buffer A, gently homogenized and layered on top of a step gradient (10 ml 50% sucrose, 26 ml 22.5% sucrose, in buffer A) for further purification. After 1 h of centrifugation at 100 000 × *g* the MAM fraction was harvested from the top of the gradient, diluted 3-fold with buffer B and centrifuged for 1 h at 100 000 × *g*. The resulting pellet was suspended in buffer B.

2.3. Characterization of subcellular fractions

Succinate dehydrogenase [13], NADPH–cytochrome-*c* reductase [14], and GDP-mannosyltransferase [2] were assayed according to published procedures. Western blot analysis using rabbit antibodies against porin (outer mitochondrial membrane), ADP/ATP-carrier (inner mitochondrial membrane), the 40 kDa microsomal protein [12], Kex2 proteinase (Golgi), Sec61 protein (endoplasmic reticulum) and phosphatidylserine synthase were carried out as described earlier [15]. Antiserum against Kex2 proteinase was kindly provided by R. Fuller, Stanford, USA, antiserum against Sec61 protein was a gift of R. Schekman, Berkeley, USA, and antibody against phosphatidylserine synthase was from G. Carman, Rutgers, USA. Immunotitrations were performed after separating proteins on 12.5% SDS-polyacrylamide gels [16] and transferring to nitrocellulose filters (Amersham). Secondary antibodies (goat-anti-rabbit antibodies, linked to alkaline phosphatase or peroxidase) were used following the manufacturer's instructions. Immunoreactive proteins on Western blots were quantitated by densitometric scanning at 500 nm using a Shimadzu dual-wavelength chromato scanner CS-930.

2.4. Analysis of phospholipid-synthesizing enzymes

Phosphatidylinositol synthase [17], phosphatidylserine synthase [11], and phosphatidylserine decarboxylase [18] were analyzed as described previously. *sn*-1,2-Diacylglycerol cholinephosphotransferase was assayed by the method of Hjelmstad and Bell [19] with the modification that incubation mixtures contained neither a detergent nor exogenous phospholipids. Glycerol-3-phosphate acyltransferase was assayed by the method of Schlossmann and Bell [20].

Phosphatidylethanolamine *N*-methyltransferase and phospholipid *N*-methyltransferase were assayed essentially as reported by Kodaki and Yamashita [21] with the following modifications. The combined activities of both enzymes were measured by monitoring methylation of endogenous phosphatidylethanolamine in the presence of 0.5 mM *S*-[*methyl*-^{3}H]adenosyl-L-methionine (final specific activity 40 mCi/mmol), 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$ and 0.5 mg/ml protein in a total volume of 0.2 ml per time point. Incubations were carried out at 30° C, and samples were taken after 30 and 60 s. Lipids were extracted with 4 ml chloroform/methanol (2:1; v/v). The organic phase was washed once with 2 ml 0.034% $MgCl_2$, once with 2 ml 2 M KCl/methanol (4:1; v/v), and once with 2 ml methanol/water/chloroform (48:47:3; per vol.).

Lipids were separated by thin-layer chromatography on silica gel 60 (Merck, Darmstadt, Germany) using chloroform/methanol/25% NH_3 (50:25:6; per vol.) as a developing solvent. Spots on thin-layer plates were scraped off, and radioactivity in phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine and phosphatidylcholine was measured by liquid scintillation counting (see below).

2.5. Import of phosphatidylserine from MAMs and other microsomal fractions into mitochondria in vitro

MAMs and other microsomal fractions (final protein concentration 0.1 mg/ml, each) isolated from the wild-type strain *Saccharomyces cerevisiae* D273-10B, and density-purified mitochondria of a revertant of the phosphatidylserine synthase deficient strain *Saccharomyces cerevisiae chol* SDK03-1A (final protein concentration 1.7 mg/ml) were mixed in a total volume of 1.8 ml 0.6 M mannitol, 10 mM Tris-HCl, pH 7.4, 0.6 mM $MnCl_2$, and 0.2 mM CDP-diacylglycerol. Radioactive phosphatidylserine was synthesized in the microsomal fractions present in the assay mixture using [^{3}H]serine (30 μCi; 170 mCi/mmol) as a precursor. Mitochondria of the *chol* revertant strain alone did not exhibit phosphatidylserine synthesis. After 10 min of incubation EDTA was added to a final concentration of 5 mM, and the incubation was continued for further 15 min. Addition of EDTA stimulates the formation of phosphatidylethanolamine by activating the cation-sensitive phosphatidylserine decarboxylase, which was used as reporter enzyme for the import of phosphatidylserine into mitochondria. Samples of 0.3 ml were taken at 0, 5, 10, 15, 20, and 25 min, the reaction was stopped by the addition of 4 ml chloroform/methanol (2:1; v/v), and lipids were extracted according to the method of Folch et al. [22] for further analyses.

2.6. Lipid analyses

Individual phospholipids were separated by thin-layer chromatography using silica gel 60 plates (Merck, Darmstadt, Germany) with chloroform/methanol/25% NH_3 (50:25:6; per vol.) as developing solvent. Spots were visualized in iodine vapor, scraped off the plate, and radioactivity was measured by liquid scintillation counting using LSC Safety (Baker) +5% water as a scintillation cocktail. Two-dimensional separation of phospholipids was performed using chloroform/methanol/25% NH_3 (65:35:5; per vol.) as developing solvent for the first direction, and chloroform/acetone/methanol/acetic acid/H_2O (50:20:10:10:5; per vol.) for the second direction. Lipid phosphorus was quantitated by the method of Broekhuyse [23].

Ergosterol was quantitated by densitometric scanning at 275 nm on a Shimadzu dual-wavelength chromato scanner CS-930 [2] after separating lipids by thin-layer chromatography using light petroleum/diethyl ether/acetic acid (80:20:2; per vol.) as a developing solvent.

2.7. Miscellaneous analytical procedures

Quantitation [24] and SDS-PAGE [16] of proteins were carried out by published procedures. Densitometric scanning of proteins after separation by SDS-gel electrophoresis and staining with Coomassie blue was performed at 540 nm using a Shimadzu dual-wavelength chromato scanner CS-930.

3. Results

3.1. Isolation and characterization of MAMs, a microsomal fraction associated with mitochondria

Yeast mitochondria prepared from spheroplasts by mild hypotonic shock and moderate mechanical force contain significant activities of several phospholipid-synthesizing enzymes, which cofractionate with the outer mitochondrial membrane [17]. On the other hand, microsomes had been demonstrated to be the major site of the biosynthesis of these phospholipids [2,17]. This discrepancy raised the question whether phospholipid-synthesizing enzymes

Table 1
Marker proteins in yeast subcellular fractions

	Relative enrichment factor						
	SDH	ADP/ATP-carrier	porin	MAT	NADPH–cytochrome-c reductase	40 kDa protein	Sec61 protein
Homogenate	1	1	1	1	1	1	1
Mitochondria (crude)	4.0	3.7	5.0	2.0	0.4	0	1.7
Mitochondria (gradient purified)	7.5	7.2	6.0	1.5	0.4	0	1.7
MAM	1.7	1.5	3.0	2.2	1.0	1.5	4.0
30 000 × *g* microsomes	–	< 0.1	0.8	3.3	2.3	2.7	4.0
40 000 × *g* microsomes	–	< 0.1	0.2	2.1	1.3	1.9	2.0
100 000 × *g* microsomes	–	< 0.1	< 0.1	0.8	0.2	1.0	1.0

SDH, succinate dehydrogenase; MAT, GDP-mannosyltransferase; 40 kDa, 40 kDa microsomal protein. Data are mean values of at least three independent experiments. –, not determined. The maximum mean deviation with Western blot analyses was 13%, with enzyme assays 10%.

coisolated reproducibly with mitochondria are 'true' mitochondrial components, or if the observed activities were actually constituents of other cellular fraction(s) associated with mitochondria. Evidence presented in this paper favors the latter explanation for reasons outlined below.

When mitochondria isolated by conventional procedures were treated with buffer at pH 6.0 and subjected to density gradient centrifugation as described in Materials and methods, a distinct microsomal compartment was released, which was named MAM (*m*icrosomes *a*ssociated with *m*itochondria) in analogy to a similar fraction described by Vance [9,25] for mammalian cells. As can be seen from Table 1 this yeast fraction differs slightly from 'classical' microsomes with respect to conventional microsomal markers (NADPH–cytochrome-*c* reductase, GDP-mannosyltransferase, or the 40 kDa microsomal protein). All these markers were found at highest enrichment in 30 000 $\times g$ microsomes; 40 000 $\times g$ microsomes and MAMs exhibited a similar enrichment pattern for the above mentioned enzymes. In contrast the Sec61 protein, a marker of the endoplasmic reticulum [26], was found equally enriched in MAMs and 30 000 $\times g$ microsomes. Appearance of mitochondrial markers (succinate dehydrogenase, porin, ADP/ATP-carrier) in the MAM fraction can be explained by cross-contamination with mitochondria or submitochondrial particles. The protein pattern obtained by SDS-PAGE (Fig. 1) of MAMs showed several similarities to that of purified mitochondria and microsomal fractions, but also some specific differences, which could more clearly be seen in a densitometric scan of the SDS-gel stained with Coomassie blue (Fig. 2). Characteristic proteins of MAMs appeared as bands with molecular weights of 30, 35, 50, 80 and 120 kDa, respectively. Other proteins could not unambiguously be distinguished from mitochondrial or microsomal proteins, which is in accordance to results obtained by determination of marker proteins of the latter two fractions.

The lipid composition of MAMs is different from that of mitochondrial and other microsomal membranes insofar as a significantly higher content of phosphatidylinositol,

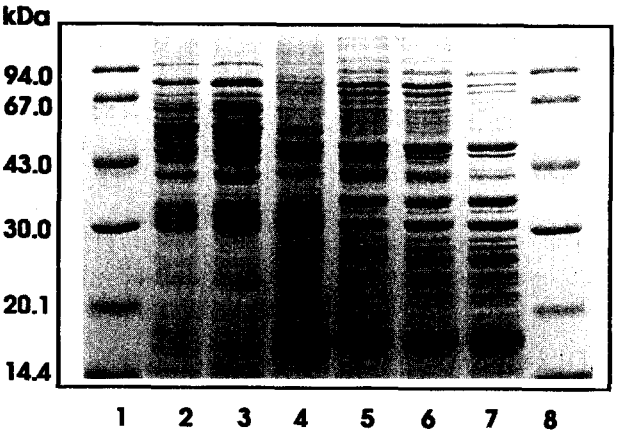

Fig. 1. The protein pattern of MAMs is distinct from that of mitochondria and other microsomal fractions. Subcellular fractions were prepared as described in Materials and methods. 30 μg protein were applied to each lane of the 12.5% SDS-polyacrylamide gel. Proteins were stained with Coomassie blue. 1 and 8: molecular weight standards (as indicated in the figure); 2: 'crude' mitochondria; 3: purified mitochondria; 4: MAM; 5: 30 000 $\times g$ microsomes; 6: 40 000 $\times g$ microsomes; 7: 100 000 $\times g$ microsomes.

and a very low concentration of phosphatidic acid were found (Table 2). Due to cross-contamination with mitochondria (see Table 1) some cardiolipin (1.7% of total phospholipids), a phospholipid characteristic for the inner mitochondrial membrane of all eukaryotes [1], was present in MAMs. The ergosterol to protein ratio is low resembling that of other internal membranes of yeast [2].

3.2. Enzymes of phospholipid biosynthesis present in MAMs

Data summarized in Table 3 show that activity of several phospholipid-synthesizing enzymes was detectable in MAMs. Two of these enzymes, namely phosphatidylserine synthase and phosphatidylinositol synthase, were found to be specifically enriched in this fraction. Immunotitrations using antisera against phosphatidylserine synthase (data not shown) exhibited results equivalent to measurements of the enzyme activity. These data suggest

Table 2
Lipid composition of MAM as compared to mitochondria and 'classical' microsomes

	% of total phospholipids			
	MAM	mitochondria	40 000 $\times g$ microsomes	100 000 $\times g$ microsomes
Phospholipid (mg/mg protein):	0.4	0.09	0.22	0.06
Ergosterol (mg/mg protein):	0.06	0.01	0.05	0.01
Phosphatidylcholine	41.5	40.2	45.2	49.6
Phosphatidylethanolamine	20.7	26.5	21.9	22.6
Phosphatidylinositol	28.9	14.6	11.4	8.9
Phosphatidylserine	6.8	3.0	8.0	9.6
Cardiolipin	1.7	13.3	1.0	0.7
Phosphatidic acid	0.4	1.4	4.1	2.3
Others	N.D.	N.D.	8.4	6.1

Data are mean values of three independent experiments. N.D., not detectable. The maximum mean deviation was 8% of individual values.

that at least some of the phospholipids destined for the import into mitochondria may indeed originate from MAMs. In purified mitochondria specific activities of all phospholipid-synthesizing enzymes tested – with the exception of the 'true' mitochondrial phosphatidylserine decarboxylase – were lower as compared to crude mitochondria, which is in line with the assumption that these residual activities resulted from cross-contamination with microsomal subfractions. Glycerophosphate acyltransferase, *sn*-1,2-diacylglycerol cholinephosphotransferase,

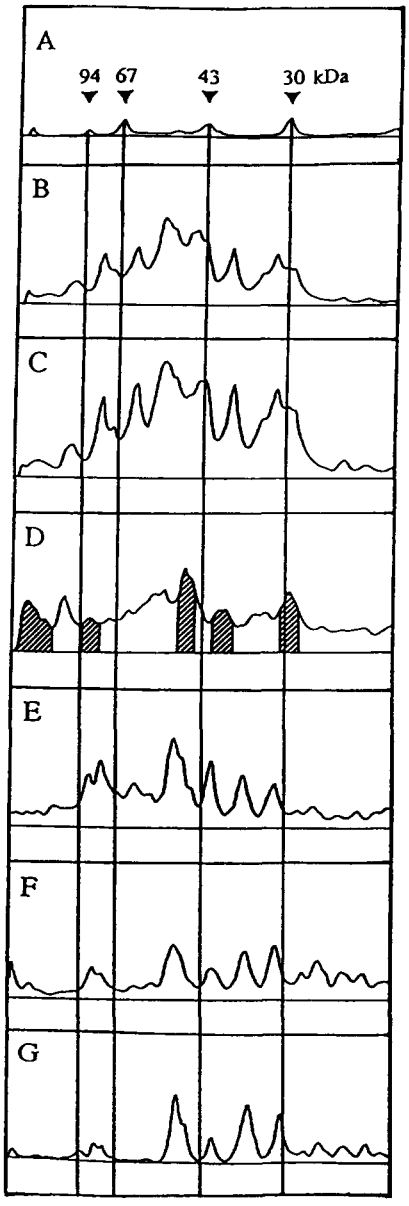

Fig. 2. Densitometric scans of protein patterns of subcellular fractions. Proteins separated by SDS-polyacrylamide gel electrophoresis were scanned as described in Materials and methods. Hatched areas indicate molecular weight ranges containing proteins specifically enriched in MAMs. (A) Standards (molecular weights as indicated in the figure); (B) 'crude' mitochondria; (C) purified mitochondria; (D) MAM; (E) 30000× *g* microsomes; (F) 40000× *g* microsomes; (G) 100000× *g* microsomes;

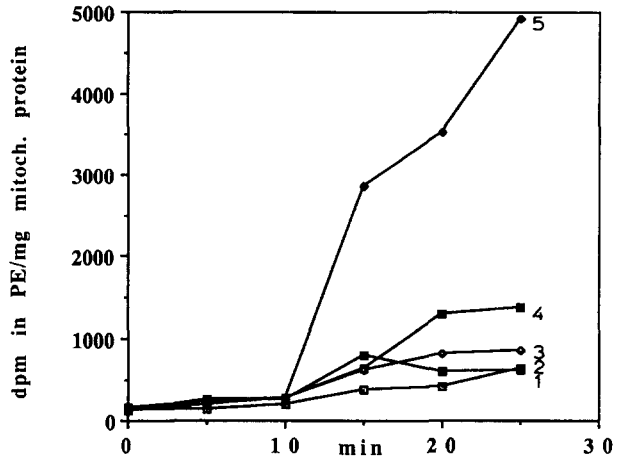

Fig. 3. Import of phosphatidylserine from MAMs and other microsomal fractions into mitochondria in a reconstituted system. Isolated MAMs, 30000, 40000 and 100000× *g* microsomes of wild-type cells were incubated with mitochondria of a phosphatidylserine synthase deficient revertant strain of *Saccharomyces cerevisiae chol* as described in more detail in Materials and methods. Data shown are from a typical experiment, that was reproduced at least three times. 1, *chol* mitochondria; 2, *chol* mitochondria + wild-type 100000× *g* microsomes; 3, *chol* mitochondria + wild-type 40000× *g* microsomes; 4, *chol* mitochondria + wild-type 30000× *g* microsomes; 5, *chol* mitochondria + wild-type MAMs.

phosphatidylethanolamine *N*-methyltransferase and phospholipid *N*-methyltransferase were also detected in the MAM fraction, but significantly higher specific activities were found in 30000 × *g* microsomes, which also contained higher concentrations of the marker proteins GDP-mannosyltransferase, NADPH–cytochrome-*c* reductase and 40 kDa microsomal protein. The enrichment factors for phosphatidylethanolamine *N*-methyltransferase and phospholipid *N*-methyltransferase in microsomal fractions were found to be relatively low. Previous work [2] had shown that a much higher enrichment of both enzymes could be obtained, when other specialized subfractions of the 30000 × *g* organelle pellet were isolated.

Taken together the results shown so far are compatible with the existence of a specific subfraction of the endoplasmic reticulum, which is associated with mitochondria, harbors certain enzymes of phospholipid biosynthesis at high specific activity, and can be distinguished from other microsomal subfractions by its protein pattern and the abundance of 'classical' microsomal markers.

3.3. Import of phosphatidylserine from MAMs into mitochondria

Association of a distinct microsomal subfraction with mitochondria raises the question as to the physiological relevance of such organellar contact. The fact that MAMs harbor phospholipid-synthesizing enzymes, especially phosphatidylserine synthase, led us to speculate, that phospholipids destined for the import into mitochondria may be

Table 3
Subcellular localization of lipid-synthesizing enzymes

	Relative enrichment (-fold)					
	PSS	PIS	GAT	CPT	PEMT	PSD
Homogenate	1	1	1	1	1	1
Mitochondria (crude)	1.1	2.1	3.0	2.5	0.9	2.4
Mitochondria (gradient purified)	0.7	1.8	2.9	1.9	0.8	4.1
MAM	5.5	5.2	2.1	2.5	0.6	0.9
30 000 × *g* microsomes	2.7	2.8	4.5	7.0	1.2	0.7
40 000 × *g* microsomes	1.6	1.7	1.5	4.5	0.9	0.6
100 000 × *g* microsomes	0.7	0.5	0.5	0.5	0.7	0.4

PSS, phosphatidylserine synthase; PIS, phosphatidylinositol synthase; GAT, glycerophosphate acyltransferase; CPT, *sn*-1,2-diacylglycerol cholinephosphotransferase; PEMT, phosphatidylethanolamine *N*-methyltransferase; PSD, phosphatidylserine decarboxylase. The maximum mean deviation was 13%.

formed in MAMs and translocated to mitochondria via membrane contact. The following strategy was designed to test this assumption. We had demonstrated before [8] that mitochondria which had not been treated at pH 6.0 and therefore had MAMs attached to them, were capable of phosphatidylserine synthesis and decarboxylation to phosphatidylethanolamine. The question arose whether this complex system of phosphatidylserine synthesis, translocation and decarboxylation could be reconstituted from its components, namely MAMs and pH 6.0 treated purified mitochondria. Reconstitution studies were complicated by the fact that mitochondria from wild-type cells cannot be prepared completely free of MAMs and thus will always have some residual phosphatidylserine synthase activity. For this reason a yeast mutant deficient in phosphatidylserine synthase, a revertant strain of *Saccharomyces cerevisiae chol* [11], was chosen for the isolation of acceptor mitochondria. These mitochondria had a very low tendency to incorporate [^{3}H]serine into phosphatidylethanolamine for reasons that are not clearly understood. When MAMs of wild-type cells were mixed with purified mitochondria of the phosphatidylserine synthase deficient revertant strain only MAMs synthesized radiolabeled phosphatidylserine with [^{3}H]serine as a precursor. Phosphatidylserine imported into mitochondria was utilized as the substrate for the mitochondrial phosphatidylserine decarboxylase. Thus the amount of newly synthesized radiolabeled phosphatidylethanolamine could serve as a measure for the import of phosphatidylserine into mitochondria. An efficient incorporation of radioactivity into phosphatidylethanolamine was observed (Fig. 3), when MAMs from wild-type cells were co-incubated with isolated *chol* mitochondria without further additives. Thus it appears that contact between MAMs and mitochondria led to phosphatidylserine translocation from the former to the latter organelle. However, contact between MAMs and mitochondria cannot be regarded as specific, at least in vitro, because also other subfractions of microsomes associate with mitochondria. Incubation of 30 000, 40 000 and 100 000 × *g* microsomes with mitochondria in the reconstitution assay described above also led to formation of phosphatidylethanolamine, although to a lesser extent. Since these microsomal fractions contain less phosphatidylserine synthase activity as compared to MAMs, this result was not surprising. The percentage of phosphatidylethanolamine formed from phosphatidylserine, however, was roughly the same with all microsomal fractions including MAMs. Preliminary experiments testing the association of microsomes with mitochondria using a binding assay similar to that described by Sturbois et al. [27] confirmed the view that there is no selectivity for organelle contact between mitochondria and MAMs in vitro (data not shown).

4. Discussion

In the present paper we report about the isolation of a yeast microsomal fraction which is associated with mitochondria (MAM). This compartment resembles in several respects 'fraction X' of higher eukaryotes described by Vance [9]. Firstly, yeast MAMs do not show the pattern of 'classical' microsomal markers (see Table 1). Nevertheless, MAMs were classified as a microsomal subfraction because of their sedimentation properties and the fact, that they contain microsomal markers at an elevated, although not at the maximum level observed in other microsomal fractions. MAMs are not enriched in Golgi membranes, because they do not react with an antibody against Kex2 proteinase [28] (data not shown). Secondly, the MAM-fraction harbors phospholipid-synthesizing enzymes. The specific activities of phosphatidylserine synthase and phosphatidylinositol synthase were markedly higher in this fraction as compared to other microsomal fractions, e.g., 30 000 × *g* microsomes (see Table 2). It is noteworthy that different phospholipid-synthesizing enzymes are differentially accumulated in MAMs. Thirdly, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine are the major phospholipids of the yeast MAM-fraction (see Table 2) as well as of the mammalian 'fraction X' [9]. The almost three times higher level of phosphatidylinositol in MAMs as compared to the other microsomal fractions is remarkable.

The protein pattern of yeast MAMs is distinct from that

of other microsomal fractions and mitochondria insofar, as several specific proteins could be detected (see Figs. 1 and 2). These findings are in accordance with results obtained by Vance [9] and Gasnier et al. [29] for the mammalian counterpart. The function of these proteins is obscure at present, but it will be a task for the future to elucidate if these proteins might be involved in the association of MAMs with mitochondria, in lipid metabolism or lipid translocation.

Membrane contact as a possible mechanism of intracellular transfer of lipids has been discussed by several authors including ourselves [4–10,25,30–32], but the experimental evidence for its physiological relevance is rare. Results describing the translocation of phosphatidylserine and phosphatidylethanolamine between the outer and the inner mitochondrial membrane are probably one of the best examples which demonstrate an involvement of membrane contact sites in lipid movement. Very recently Ardail et al. [10] reported that the mammalian 'fraction X' was found to be in close association with contact sites between the outer and the inner mitochondrial membrane thus providing an import machinery for phosphatidylserine into the mitochondrion. This observation is in line with previous findings from our laboratory [32] that preparations of yeast mitochondrial contact sites (with MAMs attached to the outer mitochondrial membrane) were able to perform concerted synthesis, transport and metabolic conversion of phosphatidylserine.

Membrane contact seems to be a reasonable prerequisite for lipid movements between organellar membranes, because direct interaction of lipophilic phases (membranes) provides a convenient environment for the translocation event, and auxiliary factors, e.g., lipid transfer proteins or transport vesicles, are not required. However, questions as to the components, which govern membrane association and temporary (?) membrane fusion and regulate the selectivity of lipid transport, remain open. Proteins located on the surface of organelles might be involved, which would parallel the situation during fusion of vesicular compartments in the process of protein secretion [33].

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