In baby hamster kidney and other cultured cells the majority of phosphatidylethanolamine (PE) is synthesized from phosphatidylserine (PS) in a process which involves transport of PS from the endoplasmic reticulum to mitochondria and decarboxylation therein by PS decarboxylase. To study the mechanism of this transport process, we first determined the molecular species composition of PE and PS from baby hamster kidney and Chinese hamster ovary cells. Interestingly, the hydrophilic diacyl molecular species were found to be much more abundant in PE than in PS, suggesting that hydrophilic PS species may be more readily transported to mitochondria than the hydrophobic ones. To study this, we compared the rates of decarboxylation of different PS molecular species in these cells. The cells were pulse labeled with [3H]serine whereafter the distribution of the labels among PS and PE molecular species was determined by reverse phase high performance liquid chromatography and liquid scintillation counting. The hydrophilic PE species contained relatively much more 3H label than those of PS, which indicates that they are more readily decarboxylated than the hydrophobic ones. Control experiments showed that differences in [3H]PS and -PE molecular species profiles are not due to (i) incorporation of 3H label to some PE species via alternative pathways, (ii) differences in degradation or remodeling among species, or (iii) selective decarboxylation of PS molecular species by the enzyme. Therefore, hydrophilic PS species are indeed decarboxylated faster than the hydrophobic ones. The rate of decarboxylation decreased systematically with hydrophobicity, strongly suggesting that formation of so-called activated monomers, i.e. lipid molecules perpendicularly displaced from the membrane (Jones, J. D., and Thompson, T. E. (1990) Biochemistry 29, 1593–1600), is the rate-limiting step in the transport of PS from the endoplasmic reticulum to mitochondria. The formation of activated monomers and thus the rate of transfer is probably greatly enhanced by frequent collisions between the two membranes which tend to be closely associated. The present data also provides a feasible explanation why hydrophilic molecular species in these cells are much more abundant in PE as compared with PS, its immediate precursor.

In many mammalian cells in culture most of phosphatidylethanolamine (PE) is synthesized from phosphatidylserine (PS) in a process which involves transfer of PS from the endoplasmic reticulum (ER) to mitochondria, decarboxylation by PS decarboxylase (PSD) located in the inner mitochondrial membrane (1) and transfer of the nascent PE back to ER (2–7). Transport of PS to mitochondria (i) is ATP-dependent (8), (ii) does not seem to require soluble cytoplasmic components, including lipid transfer proteins (8, 9) or the cytoskeleton (8), (iii) takes place only between autologous ER membrane and mitochondria (10), and (iv) involves mainly newly made PS (11, 12). Based on these facts, it has been suggested that the transport occurs upon collision of the ER and mitochondrial membranes (8, 11). Supporting this, it has been shown that fragments of ER-related membrane (MAM, mitochondria-associated membrane) enriched in PS synthase and other enzymes of lipid biosynthesis cosediment with mitochondria from hepatocytes (13), CHO cells (12), and yeast Saccharomyces cerevisiae (14). The close association of MAM with mitochondria is expected to increase the probability of productive collisions.

To further elucidate the mechanism of PS transfer from ER to mitochondria, we have here compared the rates of decarboxylation of newly synthesized PS molecular species in cultured cells. Intriguingly, the results show that the hydrophilic PS species are decarboxylated much faster than the more hydrophobic ones.

EXPERIMENTAL PROCEDURES

Reagents—The unlabeled phospholipids were supplied by Avanti Polar Lipids (Birmingham, AL) and β-chloro-L-alanine, L-cycloserine, and phospholipase C (type XI from Bacillus cereus, EC 3.1.4.3) by Sigma. The solvents were of reagent or HPLC grade and were obtained from Merck (Darmstadt, Germany), as were the TLC plates. t-[3,4-3H]Serine (27 Ci/mmol) was provided by Amersham International (Amersham, United Kingdom).

Cell Culture—BHK-21 cells were cultured on plastic tissue culture dishes in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were grown at 37 °C in a humidified atmosphere containing 5% CO2 in air.

The abbreviations used are: PE, phosphatidylethanolamine; BHK, baby hamster kidney; CHO, Chinese hamster ovary; ER, endoplasmic reticulum; MAM, mitochondria-associated membrane; nLTP, nonspecific lipid transfer protein; ODS, octadecylsilica; PS, phosphatidylserine; PSD, phosphatidylserine decarboxylase; RP-HPLC, reverse phase high performance liquid chromatography; TNP, trinitrophenyl; PBS, phosphate-buffered saline.
dishes or flasks (Nunc, Roskilde, Denmark) in Dulbecco’s modified Eagle’s medium (41965–039) supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (200 units/ml), and streptomycin (200 μg/ml) under 5% CO₂ at 37 °C. CHO-K1 cells were cultured similarly except that minimal essential α-medium (22561-021) was used instead. All cell culture media and supplements were purchased from Life Technologies, Inc. (United Kingdom).

**Labeling of PS and PE Molecular Species by [³H]Serine in Intact Cells**—Confluent monolayers of BHK or CHO cells were incubated in a serum-free medium for 12 h, washed twice with PBS, and then labeled for 15 min at 37 °C in serine-free minimal essential medium (21090-022) containing 15 μCi of [³H]serine. In some experiments, β-cholera-lamine (1 mM), an inhibitor of serine palmitoyltransferase (16) was added to the medium 2 h prior to the pulse. After the pulse, the cells were washed twice with PBS and chased at 37 °C in serum-free medium containing serine (1 mM) and ethanolamine (1 mM) for up to 22 h. They were then washed twice with PBS, scraped into PBS, pelleted by low-speed centrifugation and, after addition of bovine brain PS carrier (50 nmol/dish), the lipids were extracted according to Bligh and Dyer (17). The lipid extract was subjected to TLC using chloroform/methanol/acetate acid/water (25:15:4:1) as solvent. The bands corresponding to PS and PE were scraped off and the lipids were eluted from silica with chloroform, methanol, 0.2 M acetic acid (1:1:0.2.

To analyze the [³H] labeling of PS and PE molecular species, these lipids were converted to trinitrophenyl (TNP)-derivatives essentially as described previously (18). The yield of TNP-PE and TNP-PS was 95% and 91%, respectively. Control experiments with standards showed that there was, as expected, no differences in the degree of derivatization of individual PE or PS molecular species. The TNP-derivatives were purified by TLC using chloroform/acetone/methanol/acetate acid/water (10:4:2:0.5) as solvent and extracted from silica as above. TNP-PS and TNP-PE molecular species were then separated on an Ultrasphere 4.6 × 250-mm ODS (5 μm) column (Beckman Inc., CA) using 30 mM choline chloride in methanol, 25 mM KH₂PO₄, acetonitrile, acetic acid (90:6:2:25:9, v/v/v) (19) as solvent. Fractions were collected and their radioactivity was measured by liquid scintillation counting.

The identity of the cellular PS and PE molecular species was determined by comparing their relative retention times with TNP-PE and TNP-PS standards prepared from phosphatidylcholine species by phospholipase D-mediated transphosphatidylation (20) of the following phosphatidylcholine species: 12:0/12:0, 14:0/14:0, 15:0/15:0, 16:0/16:0, 16:0/18:1, 16:0/18:2, 16:0/18:3, 16:0/20:4, 16:0/22:6, 17:0/17:0, 18:0/18:1, 18:0/18:3, 18:0/20:4, 18:0/22:6, 18:1/18:1, and 18:2/18:2. The identification was confirmed using various other methods (see below).

**Decarboxylation of [³H]PS Species in Triton X-100 Micelles**—[³H]PS (approximately 450 nmol) was blown to dryness, 0.1 M phosphate buffer (pH 7.4) containing 1.5 mM Triton X-100 and 1 mM EDTA was added and the mixture was sonicated for 4 min on a bath sonicator to assure the complete dispersion of the lipid. After addition of crude rat liver PS (0.9 mg) solubilized (21) in the same buffer, the reaction mixture was incubated in a shaking water bath at 37 °C and aliquots were removed and mixed with chloroform/methanol (1:2) at 5, 10, 15, and 20 min of incubation. Lipids were then extracted and [³H]PS and [³H]PE were separated with TLC as described above, except that the TLC plate was first developed with acetone to remove β-mercaptoethanol (originating from the enzyme preparation) which interferes with the subsequent derivatization step.

**Determination of the Molecular Species Composition of Cellular PS and PE**—Four different methods were used to determine the molecular species composition of cellular PS and PE. First, PS and PE were converted to TNP-derivatives and the molecular species separated by reverse phase HPLC (RP-HPLC) as above and quantitated based on absorbance at 340 nm (18). Second, PE was hydrolyzed to diglycerides by incubation with phospholipase C and the diglycerides formed were converted to benzyol derivatives. These were separated into diacyl, alkylacyl, and alkylacyl subclasses, each of which was then fractionated into molecular species on a RP-HPLC column. All these steps were carried out essentially as detailed in Ref. 22. Third, the isolated PS and PE classes were subjected to electrospray mass spectroscopic analysis on a Micromass Quattro II tandem quadrupole mass spectrometer (Micromass, Altrincham, UK). This method allows both qualitative and quantitative analysis of the individual species (23). Fourth, gas chromatography analysis (24) of the fatty acid methyl esters of the total PE and PS fractions was performed.

**RESULTS**

**Molecular Species Profile of PS Is Distinct from that of PE in BHK and CHO Cells**—Fig. 1 shows the reverse phase HPLC separation of PS and PE molecular species from BHK cells. The identity of the species eluting in different...
TABLE I

| Peak | Diacyl species | Alkenyl-acyl species* |
|------|----------------|---------------------|
| 1    | 16:0/22:6      |                     |
| 2    | 16:0/20:4      |                     |
| 3    | 18:1/20:4      |                     |
| 4    | 16:0/18:2      |                     |
| 5    | 16:1/18:2      | 16:0/22:6          |
| 6    | 16:0/22:5 (n-3) | 16:0/20:4         |
| 7    | 16:0/18:1      |                     |
| 8    | 18:0/18:2      |                     |
| 9    | 18:0/18:2      |                     |
| 10   | ND             |                     |
| 11   | 18:0/22:5 (n-3) | 16:0/18:1         |
| 12   | 18:0/22:5 (n-6) | 18:0/22:6        |
| 13   | 18:0/18:1      |                     |
| 14   | 16:0/18:0      |                     |
| 15   | 18:0/18:1      |                     |

* Alkenyl-acyl species are present only in PE, where they constitute approximately 40%. PE also contains about 5% alkyl-acyl species (not shown) each of which is a very minor one.

Not detected in PS.

Not determined.

The molecular species compositions of PS and PE were determined by reverse phase HPLC analysis of the TNP-derivatives as well as with electrospray mass spectroscopy. PS and PE fractions were isolated, converted to TNP-derivatives and analyzed by RP-HPLC as specified under "Experimental Procedures." Electrospray mass spectroscopic analyses gave results consistent with the chromatographic data. The PE composition was confirmed by reverse-phase HPLC analysis of the molecular species as benzoyl diglycerides, PE composition was further studied by RP-HPLC analysis of the diacyl, alkenyl-acyl and alkyl-acyl subclasses of benzoyl derivatives (see "Experimental Procedures").

To study to which extent these differences derive from the fact that PE from BHK cells contains significant amounts of plasmalogens, TNP-PE was subjected to acid treatment to cleave the plasmalogens. As shown in Fig. 1, bottom panel, the profile is, as expected, somewhat different from that of untreated TNP-PE, but the prevalence of the hydrophilic species, as compared with PS (Fig. 1, top panel), is essentially retained. Analogous results were obtained with CHO cells (data not shown). The identity and relative abundance of PS and PE molecular species were confirmed by electrospray mass spectroscopy (23) and in the case of PE, also by RP-HPLC analysis of diacyl, alkenyl-acyl, and alkyl-acyl classes as benzoyl diglycerides (see "Experimental Procedures").

Since in BHK and CHO cells most, or even all of PE is derived from PS by decarboxylation (2–5), it is striking that the molecular species profiles of PS, the precursor, is so different from that of the product, i.e. PE. This, intriguingly, suggest that the hydrophilic PS species may be decarboxylated more rapidly than the hydrophobic ones in these cells.
Decarboxylation of Phosphatidylserine Molecular Species

The amount of radioactivity in peaks 12 and 13 (marked with an asterisk) is markedly decreased in \( \beta \)-chloinalanine-treated cells. This is reflected in the lower radioactivity in these peaks in the \( \beta \)-chloinalanine-treated samples compared to the control samples (Fig. 2, middle panel). This indicates that the hydrophilic PS species are preferentially decarboxylated in these cells. However, several alternative explanations have to be excluded before such a conclusion may eventually be drawn.

Some Minor PE Species Are Labeled from \( {\text{[3H]}} \)Serine via the Sphingosine-Phosphoethanolamine Pathway—It is known that \( {\text{[3H]}} \)serine radioactivity can incorporate to PE also via an alternative pathway that involves (i) incorporation of serine to sphingosine (ii), cleavage of sphingosine to phosphoethanolamine, (iii) formation of CDP-ethanolamine, and (iv) reaction of the CDP-ethanolamine with diglyceride to form PE (27). To assess the contribution of this pathway to labeling of PE species, the pulse-chase experiment was repeated in the presence of \( \beta \)-chloinalanine, an inhibitor of serine palmitoyltransferase (16, 28). TLC analysis showed that the fraction of \( \text{H} \) label in sphingomyelin, which was 16–19% of total lipid radioactivity in control cells, decreased to zero in the presence of \( \beta \)-chloinalanine. This compound thus effectively blocks the metabolism of \( {\text{[3H]}} \)serine via the sphingosine-phosphoethanolamine pathway in BHK cells.

Fig. 3, top panel, shows the distribution of \( \text{H} \) radioactivity among PE species in \( \beta \)-chloinalanine-treated and control cells. The amount of radioactivity in peaks 12 and 13 (marked with an asterisk) is markedly decreased in \( \beta \)-chloinalanine-treated cells. Interestingly, the label in peaks 12 and 13 also exhibited fast turnover in the pulse-chase experiment described above (Fig. 2). As expected, \( \beta \)-chloinalanine had no effect on the distribution of \( \text{H} \) label in PS (Fig. 3, bottom panel). Analogous results were obtained with cycloserine, another inhibitor of serine palmitoyltransferase (data not shown). These data show that some PE species are labeled significantly via the hydrophilic PS species. In contrast, 20.3 ± 2.3% of the \( \text{H} \) label in PE was found in the lower phase, thus demonstrating a significant labeling of the diglyceride moiety.

To determine how the diglyceride label distributes between the \( {\text{[3H]}} \)PE species, the \( {\text{[3H]}} \)diglycerides derived from PE were converted to benzoyl derivatives and separated into molecular species by RP-HPLC. As shown in Fig. 4, a major part of the label was restricted to three diacyl diglyceride peaks. Based on comparison with authentic benzoyl diglyceride standards, these peaks were found to correspond to peaks 1–4, 7, and 8, respectively, in Fig. 1, second panel from the top. These three diglyceride peaks contained 19, 13, and 17% of the total \( {\text{[3H]}} \)diglyceride label, respectively. Based on these figures and the fact that 20% of the total \( {\text{[3H]}} \)PE radioactivity was in the diglyceride moiety, it was calculated that approximately 15, 17, and 11% of the total radioactivity in peaks 1–4, 7, and 8, respectively, is not derived from \( {\text{[3H]}} \)PS. However, even when these figures are taken into account, the earlier suggestion that hydrophilic PE species are decarboxylated much more rapidly than the hydrophobic ones in BHK cells, remains valid.

Differences in \( {\text{[3H]}} \)PS and \( {\text{[3H]}} \)PE Molecular Species Profiles do Not Seem to Be Explained by Selective Degradation or Remodeling—In theory, the distinct \( \text{H} \) profiles obtained for PS and PE (Fig. 2) could result from disparate degradation or

\[ \text{PE} \]
Decarboxylation of Phosphatidylserine Molecular Species

remodeling rates of different PS and/or PE species, rather than from different rates of decarboxylation. To study this, the distribution of the $^3$H label among PS and PE molecular species was determined at 0, 0.5, 1, 2, 7, and 22 h after the pulse. As shown in Fig. 5, top panel, there is a loss of radioactivity from all PS peaks during the chase. Interestingly, however, the loss appears to be more rapid from the peaks (1–6 in particular) containing the more hydrophilic species. Correspondingly, the hydrophilic PE species appear to be labeled more rapidly than the hydrophobic ones. Peaks 12 and 13 deviate from this general pattern, but this can be fully accounted by the fact that most of the label in these peaks is not derived via decarboxylation of $^3$HPS but via another pathway (see above). When the radioactivity in the corresponding PS and PE species are summed (Fig. 5, bottom panel), the total $^3$H radioactivity is found to be essentially constant over the 22-h chase for all species, except for the sphingosine-derived species 12 and 13. The stability of the $^3$H label in PS + PE is in accordance with previous studies with BHK and CHO cells (2, 3, 12). These data strongly suggest that the marked differences in the $^3$H labeling profiles of PS and PE molecular species (Fig. 2) are not due to differences in remodeling/degradation rates of PS or PE species, but instead reflect a more rapid decarboxylation of the hydrophilic PS species. Note that the distribution of $^3$H radioactivity in Fig. 5, bottom panel, indicates the relative proportions of different PS molecular species synthesized in BHK cells.

**The Kinetics of PS Decarboxylation Has Two Components**—Fig. 6 shows the decay of radioactivity for different $^3$HPS peaks, excluding those in which a major fraction of the radioactivity was not derived via $^3$HPS decarboxylation. Note that some peaks that are minor or not well separated have been grouped together. The plots indicate that in each case there are apparently two decay components, a fast and a slow one. However, the fractional contribution of these components (indicated in figure panels) varies significantly. For instance, the fast component accounts for 20, 12, and 2.5% of the total decay in peak groups 1–4, 7–9, and 14–15, respectively. Also the half-time of the slow component varies significantly being approximately 10, 18, and 32 h, respectively. The half-time of the fast component could not be determined for all species due to the limited number of data points in the relevant region. We conclude that there are two, kinetically distinct pools of PS molecules in BHK cells: one which consists predominantly of the more hydrophilic PS species and is characterized by a rapid conversion to PE, and another, consisting mostly of the more hydrophobic species and characterized by a much slower conversion to PE. This conclusion is consistent with previous studies suggesting, in a more qualitative manner, the presence of two pools of PS with different decarboxylation kinetics in cultured cells (3, 11, 12, 31).

**PS Decarboxylase Does Not Discriminate between $^3$HPS Molecular Species**—To exclude the possibility that the more efficient decarboxylation of the hydrophilic PS species simply reflects selectivity of the PSD enzyme, rather than a more rapid transfer to mitochondria, the total $^3$HPS fraction was isolated from BHK cells incubated with Triton X-100 micelles and incubated with solubilized PSD from rat liver\(^2\) for up to 20

\(\text{RRT}\), relative retention time.

---

\(^2\) Rat liver enzyme was used, because the low specific activity of the $^3$HPS available required more PSD activity than what was feasible to obtain from BHK or CHO cells. It is, however, very likely that PSD from rat behaves similarly to PSD from hamster, a closely related species.
min at 37 °C. The [3H]PS and [3H]PE profiles were then analyzed by RP-HPLC as above. As shown in Fig. 7, the [3H]PE profile is now very similar to that of [3H]PS, thus strongly suggesting that PSD does not act on some PS species preferentially. There is somewhat more radioactivity associated with the most hydrophilic PE species as compared with PS. This could be due to that these hydrophilic [3H]PS species, because of higher solubility to the aqueous phase, equilibrate more rapidly between micelles containing PSD and those devoid of it, and are thus more rapidly decarboxylated. Such hydrophobicity-dependent transfer of phospholipids between micelles has been demonstrated (32). Also supporting the absence of PSD selectivity, the distribution of radioactivity among PS and PE species remained essentially constant at all times when the decarboxylation progressed from 0 to 50% (not shown). Nevertheless, the differences between [3H]PS and [3H]PE profiles seen in Fig. 7 are minor as compared with what was observed with intact cells (Fig. 2).

**DISCUSSION**

The present study shows that hydrophilic molecular species are much more abundant in PE as compared with PS, its immediate precursor, in BHK and CHO cells. Pulse-chase experiments with [3H]serine revealed that the hydrophilic PE species contained proportionally much more label than those of PS. These findings strongly suggested that the hydrophilic PS species are decarboxylated considerably faster than the hydrophobic ones in intact cells. This could be confirmed by excluding alternative explanations, including: (i) labeling of particular PE species via the sphingosine-phosphoethanolamine pathway, (ii) selective incorporation of radioactive [3H]serine metabolites to the diglyceride moiety of some PE species, (iii) differential remodeling/degradation of PS/PE species, or (iv) selectivity of the decarboxylase enzyme itself. In addition, the possibility that [3H]ethanolamine released from [3H]PE would have reincorporated via the CDP-ethanolamine pathway to PE species seems very unlikely because the chase medium contained a large excess of unlabeled ethanolamine and second, an insignificant amount of radioactivity was associated with the plasmalogen PE species (cf., Figs. 1–3) which are synthesized via the CDP-ethanolamine pathway (33).

Why would the rate of decarboxylation of a newly synthesized PS molecule in the cell depend on its hydrophobicity? There is strong evidence that the rate of transport to mitochondria, rather than decarboxylation itself, determines the overall rate of PS decarboxylation in cells (15). Therefore, we suggest that transport of PS molecular species from their site of their

---

**FIG. 6. Analysis of the decarboxylation kinetics of PS species.** The fraction of [3H] radioactivity in PS calculated from an experiment similar to the one depicted in Fig. 5 is plotted as a function of chase time. The data was analyzed by employing the two-exponential decay model, as it gave significantly better fits than the single exponential model. (The goodness of fit, R², obtained was 0.994, 0.999, 0.934, and 1.000, respectively.) The fractional contributions and half-times obtained for the fast (T1) and slow (T2) decay components are shown in the panels. For some peaks the half-time of the fast component could not be determined due to paucity of relevant data points. **Upper panel,** peaks 1–4; **upper middle panel,** peaks 7–9; **lower middle panel,** peaks 10–11; **lower panel,** peaks 14–15. Analogous data were obtained when the experiment was repeated under similar conditions.

---

**FIG. 7. Decarboxylation of [3H]PS molecular species in Triton X-100 micelles.** The total BHK-[3H]PS fraction labeled from [3H]serine was incubated with PS decarboxylase from rat liver in the presence of Triton X-100 for 10 min. At this time 29% of the total [3H]PS pool had been converted to [3H]PE. [3H]PS (closed symbols) and [3H]PE (open symbols) molecular species were then separated and analyzed for radioactivity as indicated in the legend of Fig. 2. [3H]PE counts/min have been multiplied by 1.3 to ease the comparison of the profiles. The close similarity of the [3H]PS and -PE profiles were maintained in the samples taken after 15 or 20 min of incubation, when 43 and 50% of PS had been decarboxylated, respectively. The experiment was repeated twice with similar results. RRT, relative retention time.
Decarboxylation of Phosphatidylserine Molecular Species

The half-time of PS species decarboxylation were obtained by fitting the standard single-exponential decay model to the data shown in Fig. 6. A single-exponential model was used here instead of a two-exponential one, since it approximates better the overall rate of decarboxylation. Again, some minor and/or closely eluting peaks were combined to improve statistics, and the peaks containing species significantly labeled via the sphingosine-phosphoethanolamine pathway (Fig. 3) were omitted from the analysis. The relative reverse phase retention time (RRT) was used as the measure of species hydrophobicity (39).

This result may seem unexpected in the view that transport of natural phospholipids by such spontaneous efflux mechanism is generally considered as a very slow process (half-time tens of hours). However, it should be noted that formation of activated monomers, and hence the rate of translocation, can increase remarkably at higher membrane concentrations. Thus, the half-time for the transfer of palmitoyloleoyl-phosphatidylcholine between lipid vesicles decreased from 46 to 7 h when the acceptor vesicle concentration was increased from 2.5 to 40 mM (34). The latter half-time is similar to that for the transport of newly synthesized PS from ER to mitochondria (40, 41, this study). The enhancement of transfer at higher membrane concentrations has been attributed to stabilization of the transition state monomers by their interaction with the acceptor membrane (34). In this respect, it is noteworthy that according to morphological (42, 43) and biochemical studies (13, 44) the ER (MAM) and the outer mitochondrial membranes are closely associated. This means that the local concentration of the donor and acceptor membranes is very high, thus favoring collision-enhanced intermembrane lipid transfer. It should also be noted that the ER (MAM) and the mitochondrial outer membrane are relatively rich in PE (13, 45), and this lipid greatly enhances lipid translocation via the collisional mechanism (46). The involvement of membrane collisions from ER to mitochondria has been proposed previously (8, 11).

An alternative model for collisional lipid transfer suggests that a transient fusion or hemifusion of the two membranes occurs upon collision of membranes, thus allowing the lipid mixing by lateral diffusion (47). Since lateral diffusion is essentially independent of lipid hydrophobicity (48), this model is clearly not compatible with the present data showing marked dependence of the translocation rate on lipid hydrophobicity (Fig. 8). The absence of cardiolipin, a major component of the outer mitochondrial membrane (45), from the ER is also incompatible with this model. In contrast, the absence of cardiolipin from ER is fully consistent with the transition state monomer model, since desorption of this lipid with four acyl chains from a membrane is predicted to be an extremely slow process (38).

Although the present data are in good accordance with spontaneous, collision-enhanced transfer of PS from ER to mitochondria, they by no means preclude the involvement of proteins. In this respect, it is of interest that the rate of transport mediated by the non-specific lipid transfer protein (ns-LTP) is negatively correlated with the hydrophobicity of the lipid substrate (49, 50). This protein has been found to be associated with isolated mitochondria (51) and detected immunologically in mitochondria of intact cells (52–54). This protein also enhances transfer of PS in vitro (15, 55, 56) and is capable of net transfer of lipids (57). On the other hand, several studies have indicated that ns-LTP may not be involved in transport of PS to mitochondria, the possibility remained that the mutant compensates for the loss of ns-LTP by synthesizing more of the hydrophilic PS species which would translocate more readily via the non-mediated process (see above). Our preliminary compositional and [3H]serine labeling studies with the same CHO mutant provide evidence that such compensatory changes in PS molecular species distribution synthesis may indeed take place. Although it is not clear if these changes are related to PS transport from ER to mitochondria, further studies seem to be required to decide whether or not ns-LTP plays a role in PS transport to mitochondria.

We cannot presently exclude the possibility that the disparate decarboxylation efficiencies of different PS species is due to the presence of two or more distinct compartments of newly synthesized PS molecules in the cell. Thus, there could be a compartment close to mitochondria (e.g. MAM) where mainly hydrophilic PS species would be synthesized, and another more distant compartment where the more hydrophobic species would be produced. If so, preferential decarboxylation of the hydrophilic species could simply reflect their more favorable location regarding transport to mitochondria. We consider, 3 While acidic lipids tend to eliminate the effect of PE in vesicle systems, there may be areas of high PE and low acidic lipid content in the two membranes due to lipid domain formation; or the negative charges of lipids are neutralized by positively charged protein residues of other compounds (46).
however, this model improbable for two reasons. First, PS synthesis appears to be largely confined to the ER in rat liver (41, 59). Hence, those putative compartments should represent different regions of ER, e.g. MAM and the bulk ER. However, as the ER membrane is obviously continuous and the lateral diffusion of lipids very fast, it is difficult to see why rapid mixing of the PS species synthesized in those two regions would not take place. Such mixing is expected to be complete within a few minutes. Considering that the half-time for the transport of newly synthesized PS from ER to mitochondria is several hours (40, 41, this study), this two-compartment model could not explain the observed differences in the rate of decarboxylation between PS molecular species. Second, the data given in Fig. 8 would be compatible with this model only if one assumed that equilibration of PS species is strictly dependent on their hydrophobicity. This does not seem feasible considering that lateral diffusion of lipids is only very weakly dependent on their structure (48).

Previous studies have shown that newly synthesized PS molecules are decarboxylated much more efficiently than the pre-existing ones, thus suggesting the presence of a least two metabolically distinct pools of PS (11, 12). This is strongly supported by the present study showing that the kinetics of decarboxylation of PS species has two components: a fast one with a half-time of about 20 min and a slow one with a half-time of 10–32 h (Fig. 6). A feasible explanation for the existence of these kinetic components would be that efficient translocation of PS molecules from ER (or MAM) to mitochondria can occur only for a relatively short period of time after their synthesis. Based on the data shown in Fig. 6 we suggest that the width of this time window is 10–20 min. This time is probably determined by the rate of PS removal from the ER membrane in transport vesicles which are constantly budding from the ER membrane. The half-time of transport of phospholipids from the ER is approximately 10 min (60). For the total PS pool, the half-time of the fast and slow components were determined to be 22 min and 28 h, respectively, and approximately 12% of the total pool of the newly synthesized PS molecules was estimated to be transported to mitochondria during the fast process in BHK cells. The latter value is in accordance with the conclusion that only a small fraction of the total PS pool is turning over rapidly in hetapatocytes (31). Eventually, however, most or even all of PS synthesized may be eventually, however, most or even all of PS synthesized may be transported to mitochondria. The half-time of transport of phospholipids from the ER membrane is obviously continuous and the lateral diffusion of lipids is only very weakly dependent on their structure (48).

A major fraction of the PS removed from the ER in transport vesicles will probably end up to the plasma membrane. A recent investigation indicated that almost 70% of the total PS pool in BHK cells is located in the plasma membrane, and about 35% of the inner leaflet phospholipid is PS (61). This remarkable enrichment to the plasma membrane indicates that PS cannot move readily from this site to mitochondria. Supporting this, the movement of PS from the plasma membrane to mitochondria is an energy-dependent process (62), suggesting that diffusion through the cytoplasm is the mechanism of transport. We propose that the slow component of decarboxylation observed here (Fig. 6) represent translocation of plasma membrane PS to the mitochondria by such spontaneous diffusion. The apparent increase in half-time of this slower process with increasing PS hydrophobicity supports this mechanism. In addition, we have recently observed that transport of pyrene-labeled PS species from the plasma membrane to mitochondria in BHK cells decreases logarithmically with increasing species hydrophobicity so that for the most hydrophobic ones hardly any transfer was detectable. It is tempting to speculate that the high average hydrophobicity of PS species is physiologically relevant. It may allow the cell to maintain a high concentration of this lipid in the plasma membrane where it could be needed for such crucial processes as exo- and endocytosis (63).

It has been amply demonstrated that transport of PS from the ER to mitochondria is an ATP-dependent process (8, 10, 15, 58). ATP is probably required to sequester Ca\(^{2+}\) to a compartment in the ER, apparently to support PS synthesis which requires relatively high levels of Ca\(^{2+}\) (64). However, it appears that ATP is required also for the translocation process, possibly to bring the MAM and the outer mitochondrial membrane more closely together (58) thereby enhancing the probability of collision and thus lipid translocation.

There is good evidence that a major fraction of PE derived from PS via decarboxylation in mitochondria is transported rapidly back to ER (11, 40). The mechanism of this transfer is not known. However, if PS is transferred between ER and mitochondrial membranes via the activated monomer-collision process as suggested above, it is logical to assume that the same mechanism is employed for translocation of PE in the opposite direction. The prevalence of hydrophilic species in PE derived from PS in mitochondria (see above) should obviously be advantageous for this process to occur efficiently. Supporting a common transport mechanism for PS and PE, movement of newly made PE from mitochondria to ER is also ATP-dependent as indicated by studies with yeast (65).

In conclusion, the present data strongly suggest that hydrophilic PS species are more rapidly decarboxylated, and thus also transported to mitochondria after the synthesis in ER than the hydrophobic ones. This is best explained by assuming that formation of so-called activated lipid monomers, enhanced by intermembrane collisions, is the rate-limiting step in the transfer process. This mechanism explains why hydrophobic species are much more prominent in PE as compared with PS, its immediate precursor. Preferential conversion of the hydrophilic PS species to PE also means that the average hydrophobicity of the remaining PS species will be significantly higher than that of those originally synthesized. This could be important for the maintenance of high PS concentrations in the inner leaflet of the plasma membrane.

Acknowledgments—We are grateful to Dr. Renata Jasinska for providing the solubilized decarboxylase, Dr. Karel Wirtz for providing the CHO cells, Dr. Risto Kostianen and Juha Kokkonen for recording the mass spectra, Lic. Tech. Anu Kettunen for assistance in the kinetic analyses, and Tarja Grundström for expert technical assistance.

REFERENCES
1. Zborowski, J., Dygas, A., and Wojtzeak, L. (1983) FEBS Lett. 157, 179–182
2. Voelker, D. R. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 2669–2673
3. Miller, M. A., and Kent, C. (1986) J. Biol. Chem. 261, 9753–9761
4. Kuge, O., Nishijima, M., and Akamatsu, Y. (1986) J. Biol. Chem. 261, 5790–5794
5. Kuge, O., Nishijima, M., and Akamatsu, Y. (1986) J. Biol. Chem. 261, 5706–5709
6. Vance, J. E. (1988) Biochim. Biophys. Acta 963, 70–81
7. Arthur, G., and Lu, X. (1993) Biochem. J. 293, 125–130
8. Voelker, D. R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9921–9925
9. van Heuenden, G. P. H., Bos, K., Raetz, C. R. H., and Wirtz, K. W. A. (1990) J. Biol. Chem. 265, 4105–4110
10. Voelker, D. R. (1989) J. Biol. Chem. 264, 7069–7074
11. Vance, J. E. (1991) J. Biol. Chem. 266, 89–97
12. Shiao, Y.-J., Iwgap, G., and Vance, J. E. (1995) J. Biol. Chem. 270, 11190–11198
13. Vance, J. E. (1996) J. Biol. Chem. 271, 7248–7256
14. Zener, E., Sperka-Gottild, C. D., M. Fuchs, E. V., Kohlnhein, S. D., Paltauf, F., and Daum, G. (1991) J. Bacteriol. 173, 2039–2046
15. Voelker, D. R. (1989) J. Biol. Chem. 264, 8019–8025
16. Medlock, K. A., and Merrill, A. H., Jr. (1988) Biochemistry 27, 7079–7084
17. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
18. Hullin, F., Kim, H.-Y., and Salem, N., Jr. (1989) J. Lipid Res. 30, 1963–1975

\[4\] L. Heikinheimo and P. Somerharju, unpublished results.
Decarboxylation of Phosphatidylserine Molecular Species

3335

19. Patton, G. M., Fasulo, J. M., and Robins, S. J. (1982) *J. Lipid Res.* 23, 190–196
20. Comfurius, P., Bevers, E. M., and Zwaal, R. F. A. (1990) *J. Lipid Res.* 31, 1719–1721
21. Dygas, A., and Zborowski, J. (1989) *Acta Biochim. Polon.* 36, 131–141
22. Patton, G. M., and Robins, S. J. (1990) *Methods Enzymol.* 187, 195–215
23. Han, X., and Gross, R. W. (1995) *J. Am. Soc. Mass Spectrom.* 6, 1202–1210
24. Christie, W. C. (1989) *Gas Chromatography and Lipids. A Practical Guide*, The Oily Press, Ayr, Scotland
25. Samborski, R. W., and Vance, D. E. (1990) *Biochim. Biophys. Acta* 1167, 15–21
26. Shiao, Y.-J., and Vance, J. E. (1995) *Biochim. Biophys. Acta* 1234, 274–280
27. Hanada, K., Nishijima, M., Kiso, M., Hasegawa, A., Fujita, S., Ogawa, T., and Akamatsu, Y. (1992) *J. Biol. Chem.* 267, 23527–23533
28. Chen, H., Born, E., Mathur, S. N., and Field, F. J. (1993) *J. Lipid Res.* 34, 2159–2167
29. Xu, Z., Byers, D. M., Palmer, F. B. S., Spence, M. W., and Cook, H. W. (1991) *Biochim. Biophys. Acta* 1093, 2143–2150
30. Vance, J. E., and Vance, D. E. (1986) *J. Biol. Chem.* 261, 4486–4491
31. Bjerve, K. S. (1985) *Biochim. Biophys. Acta* 883, 396–405
32. Nichols, J. W. (1988) *Biochemistry* 27, 3925–3931
33. Jones, J. D., and Thompson, T. E. (1990) *Biochemistry* 29, 1593–1600
34. Pownall, H. J., Bick, D. L., and Massey, J. B. (1991) *Biochemistry* 30, 5696–5700
35. Massy, J. B., Gotto, A. M., Jr., and Pownall, H. J. (1982) *Biochemistry* 21, 3630–3636
36. Ferrell, J. E., Jr., Lee, K.-J., and Huestis, W. H. (1985) *Biochemistry* 24, 2857–2864
37. Silvius, J. R., and Leventis, R. (1993) *Biochemistry* 32, 13318–13326
38. Massy, J. B., Hickson, D., She, H. S., Sparrow, J. T., Via, D. P., Gotto, A. M., Jr., and Pownall, H. J. (1984) *Biochim. Biophys. Acta* 794, 274–280
39. Voelker, D. R. (1985) *J. Biol. Chem.* 260, 14671–14676
40. Vance, J. E., and Vance, D. E. (1988) *J. Biol. Chem.* 263, 5898–5909
41. Franke, W. W., and Kartenbach, J. (1971) *Protoplasma* 73, 35–41
42. Ardail, D., Gasnier, F., Lermne, F., Simonot, C., Louilot, P., and Gateau-Roesch, O. (1993) *J. Biol. Chem.* 268, 25985–25992
43. Ardail, D., Gasnier, F., Lermne, F., Simonot, C., Louilot, P., and Gateau-Roesch, O. (1993) *J. Biol. Chem.* 268, 25985–25992
44. Gaigg, B., Simberni, R., Hrstnik, C., Paltauf, F., and Daum, G. (1995) *Biochim. Biophys. Acta* 1234, 214–220
45. Hovius, R., Lambrechts, H., Nicolay, K., and de Kruijff, B. (1990) *Biochim. Biophys. Acta* 1021, 217–226
46. Winley, W. C., and Thompson, T. E. (1991) *Biochemistry* 30, 1702–1709
47. Gurd, F. R. N. (1960) in *Lipid Chemistry* (Hanahan, D. J., ed), pp. 208–259, Wiley, New York
48. Derko, Z., and Jacobson, K. (1980) *Biochemistry* 19, 6050–6057
49. Nickols, J. W., and Pagano, R. E. (1983) *J. Biol. Chem.* 258, 5368–5371
50. van Amerongen, A., Demel, R. A., Westerman, J., and Wirtz, K. W. A. (1989) *Biochim. Biophys. Acta* 1004, 36–43
51. Megli, F. M., De Lisi, A., van Amerongen, A., Wirtz, K. W. A., and Quagliariello, E. (1986) *Biochim. Biophys. Acta* 861, 463–470
52. Keller, G. A., Scallen, T. J., Clarke, D., Maher, P. A., Krisans, S. K., and Singer, S. J. (1989) *J. Cell Biol.* 108, 1353–1361
53. van Haren, L., Teerds, K. J., Osseendt, B. C., van Heusden, G. P. H., Orly, J., Stooco, D. M., Wirtz, K. W. A., and Rommerts, F. F. G. (1992) *Biochim. Biophys. Acta* 1124, 288–296
54. Mendoza-Handagama, S. M. L. C., Aten, R. F., Watkins, P. A., Scallen, T. J., and Berhan, H. R. (1995) *Tissue & Cell* 27, 483–490
55. Holvius, R., Faber, B., Brigot, B., Nicolay, K., and de Kruijff, B. (1992) *J. Biol. Chem.* 267, 16790–16795
56. Jasinska, R., Zborowski, J., and Sumerharju, P. (1993) *Biochim. Biophys. Acta* 1152, 161–170
57. Crain, R. C., and Zilversmit, D. B. (1980) *Biochim. Biophys. Acta* 620, 37–48
58. Voelker, D. R. (1990) *J. Biol. Chem.* 265, 14340–14346
59. Dennis, E. A., and Kennedy, E. P. (1972) *J. Biol. Chem.* 247, 263–267
60. Wieland, F. T., Gleason, M. L., Serafini, T. A., and Rothman, J. E. (1987) *Cell* 50, 289–300
61. Allan, D. (1986) *Mol. Membr. Biol.* 13, 81–84
62. Kobayashi, T., and Arakawa, Y. (1991) *J. Cell Biol.* 113, 235–244
63. Devaux, P. F. (1991) *Biochemistry* 30, 1163–1173
64. Borkenhagen, L. F., Kennedy, E. P., and Fielding, L. (1961) *J. Biol. Chem.* 236, 1152–1157
65. Achleitner, G., Zweytick, D., Trotter, P. J., Voelker, D. R., and Daum, G. (1995) *J. Biol. Chem.* 270, 29836–29842
Preferential Decarboxylation of Hydrophilic Phosphatidylserine Species in Cultured Cells: IMPLICATIONS ON THE MECHANISM OF TRANSPORT TO MITOCHONDRIA AND CELLULAR AMINOPHOSPHOLIPID SPECIES COMPOSITIONS

Liisa Heikinheimo and Pentti Somerharju

J. Biol. Chem. 1998, 273:3327-3335.
doi: 10.1074/jbc.273.6.3327

Access the most updated version of this article at http://www.jbc.org/content/273/6/3327

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 63 references, 33 of which can be accessed free at http://www.jbc.org/content/273/6/3327.full.html#ref-list-1