Phosphatidylserine biosynthesis in mitochondria from the Morris 7777 hepatoma

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Abstract Mitochondria from the 7777 hepatoma incorporate substantial amounts of L-[U-^{14}C]serine into phospholipid by a Ca^{2+}-dependent base-exchange reaction. This reaction is virtually absent in normal liver mitochondria. The finding cannot be attributed to microsomal contamination of the sucrose gradient-purified 7777 hepatoma mitochondria. The reaction is also absent in the rapid-growth controls, fetal rat liver and regenerating rat liver. [^{14}C]Serine incorporation into 7777 hepatoma mitochondrial phospholipid by base-exchange requires Ca^{2+} and is inhibited by EDTA. Ca^{2+} cannot be replaced by Mg^{2+}, Mn^{2+}, or Co^{2+}. The reaction is inhibited by a sulfhydryl reagent and by detergents and is abolished by heating to 70°C for 10 min. Product analysis indicates that phosphatidylserine and its decarboxylation product, phosphatidylethanolamine, are formed by 7777 hepatoma mitochondria, while phosphatidylserine is the sole product with microsomes. The conversion of phosphatidylserine into phosphatidylethanolamine in 7777 hepatoma mitochondria is inhibited by KCN. This study provides further evidence of abnormal mitochondrial biogenesis in the 7777 hepatoma. Our earlier study indicated a greatly increased mitochondrial activity of CTP:phosphatidate cytidylyltransferase in the 7777 hepatoma (Hostetler, Zenner, and Morris. 1978. *J. Lipid Res.* **19:** 553–560). The presence in mitochondria of these two enzymes, which are primarily microsomal in normal liver, does not appear to be due to rapid growth alone, since their intracellular distribution was not altered in fetal or regenerating rat liver.—**Hostetler, K. Y., B. D. Zenner, and H. P. Morris.** Phosphatidylserine biosynthesis in mitochondria from the Morris 7777 hepatoma. *J. Lipid Res.* 1979. **20:** 607–613.

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In mammalian liver, phosphatidylserine is formed by a base-exchange reaction which is calcium-dependent and does not require high-energy intermediates (1–3). Several studies of phosphatidylserine formation have indicated that this reaction is predominately microsomal (4, 5). Recently, van Golde et al. (6) showed that the small amount of activity in a well-characterized mitochondrial fraction could be accounted for by contamination with microsomes. Thus, most authorities agree that normal liver mitochondria are essentially devoid of phosphatidylserine synthesis by base-exchange. Phosphatidylserine decarboxylase, however, is present in mitochondria (4, 6). Some evidence has been presented suggesting that a cytoplasmic factor is present in liver that promotes the transfer of a phosphatidylserine from microsomes to mitochondria (7, 8).

In a previous publication, we reported that mitochondria from the 7777 hepatoma have a greatly increased phospholipid content per milligram of protein (9) and this finding has recently been confirmed by Reitz, Thompson, and Morris (10). The 75% increase in the 7777 hepatoma mitochondrial phospholipid content is not due to a decrease in the amount of protein per mitochondrion (11).

In an attempt to establish the molecular basis for the increased phospholipid content of 7777 hepatoma mitochondria, we have examined the reactions of de novo phospholipid synthesis in mitochondria. The activity of CTP: phosphatidate cytidylyltransferase (EC 2.7.7.41) was greatly increased in 7777 hepatoma mitochondria (12), particularly in the outer mitochondrial membrane (11), and was greatly reduced in the tumor microsomes (11, 12) relative to the activities found in the respective normal liver fractions. However, the localization and activity of enzymes responsible for the synthesis of phosphatidate, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylcholine did not differ greatly from those found in normal liver mitochondria and microsomes (11). The subcellular localization in CTP:phosphatidate cytidylyltransferase was normal in fetal rat liver, suggesting that malignant transformation, rather than rapid growth, might lead to defective membrane assembly, resulting in the abnormal subcellular localization of this enzyme which is rate-determining in de novo acidic phospholipid biosynthesis. Finally, no difference was noted in the degradation of endogenous [^{32}P]diphosphatidylglycerol or [^{32}P]phosphatidylcho-

line by mitochondrial phospholipase A_2 in normal liver or the 7777 hepatoma (11). Thus, the increased mitochondrial phospholipid content in the 7777 hepatoma is not likely to arise from defective catabolism.

Although the findings noted above are probably adequate to explain the moderate increase in mitochondrial diphosphatidylglycerol in the 7777 hepatoma, other explanations must be sought to explain the increased content of the major mitochondrial phosphoglycerides, phosphatidylcholine and phosphatidylethanolamine. As noted above, mitochondria from both normal liver and 7777 hepatoma are devoid of CDP-choline: *sn*-1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) (11).

In this publication, we report evidence that demonstrates that mitochondria from the 7777 hepatoma can form phosphatidylserine from L-[U-^{14}C]serine in a base-exchange reaction which is dependent on calcium. The product which accumulates in the 7777 mitochondria is phosphatidylethanolamine, formed by decarboxylation of phosphatidylserine. In agreement with the previous work of others (4–6), the formation of phosphatidylserine is essentially absent in normal liver mitochondria. Evidence is presented showing that the pathway is also absent in mitochondria from regenerating rat liver and fetal rat liver.

MATERIALS AND METHODS

Preparation and characterization of subcellular fractions

The 7777 hepatomas were maintained in male rats of the Buffalo strain by intramuscular injections of a concentrated dispersion of viable tumor cells at approximate intervals of 4–5 weeks. Livers were obtained from 18- to 20-day-old fetuses removed from pregnant female rats of the Buffalo strain. Regenerating rat liver was prepared according to the method of Higgins and Anderson (13). Fasted rats were anesthetized with ether and the large median and left lateral lobes were ligated and removed. The incision was closed and the rats were allowed free access to water containing 10% dextrose and Purina rat chow. Regenerating liver was removed 42 hr after partial hepatectomy.

Subcellular fractions were prepared from normal liver, 7777 hepatoma tissue, fetal rat liver, and regenerating rat liver as previously described. Briefly, the respective tissues were removed, minced, washed, and homogenized in ice-cold 0.25 M sucrose containing 5 mM Tris-HCl (pH 7.4) and 2 mM EDTA. Nuclei and debris were sedimented by centrifugation at 8,600 *g* for 10 min. The mitochondrial pellet was washed thrice with 0.25 M sucrose containing 5 mM Tris-HCl, pH 7.4, applied to a continuous sucrose gradient, and centrifuged at 27,000 *g* for 30 min. The mitochondrial band was harvested with a curved needle, diluted with an equal volume of distilled water, and pelleted as previously described (9). The post mitochondrial supernatant was centrifuged at 17,000 *g* for 15 min to obtain the composite pellet; the supernatant from this step was centrifuged at 105,000 *g* for 60 min and the microsomal pellet obtained was washed once with 0.25 M sucrose containing 5 mM Tris-HCl, pH 7.4. The gradient-purified mitochondria and the microsomes were dispersed in 0.25 M sucrose–5 mM Tris-HCl, pH 7.4, and stored frozen at −60°C until use. Protein was measured by the method of Lowry et al. (14). Succinate dehydrogenase activity was measured as a mitochondrial marker according to the method of Green, Mii, and Kohout (15). Rotenone-insensitive NADPH cytochrome *c* reductase was determined according to the method of Sottacasa et al. (16).

Assay of phosphatidylserine synthesis

Phosphatidylserine biosynthesis was measured in an incubation mixture containing 50 mM Tris-HCl, pH 7.4; 1.5 mM L-[U-^{14}C]serine (sp act 4.63 mCi/mmol), 10 mM $CaCl_2$, and between 0.3 and 3 mg/ml protein. A typical incubation consisted of 300 μl total volume containing 0.55 mg of protein. Incubations were carried out for 20 min at 37°C, unless otherwise noted. Reactions were stopped by extracting the entire incubation mixture with 20 volumes of chloroform–methanol 2:1 (v/v). These extractions were carried out according to the method of Folch, Lees, and Sloane Stanley (17). The lower phases of the washed extracts were removed, taken to dryness, and redissolved in 1 ml of chloroform–methanol 2:1. Aliquots (100 μl) were then removed, dried in liquid scintillation vials, and counted in a Searle Mark III liquid scintillation counter, using 15 ml of 0.5% 2,5-diphenyloxazole and 0.04% *p*-bis-(2-[5-phenyloxazole])benzene in toluene–Triton X-100–water 2:1:0.2 (by volume). Quench corrections were made using an external standard technique. In some experiments, the remaining 900 μl of the washed chloroform–methanol extracts were taken to dryness with a nitrogen stream and applied to 0.25-mm thin layers of silica gel G prepared with 0.4 M boric acid, and the chromatograms were developed with chloroform–methanol–water–concentrated ammonia 65:30:3:3 (by volume) (System A). The plates were scanned for location of the radioactive spots using a radioscanner (Panax

Equipment Ltd., Redhill, Surrey, England). Lipid spots were also visualized with iodine vapors and compared with authentic reference phospholipids. The areas on the plates corresponding to the radioactive peaks on the scans were scraped into scintillation vials and counted as described above. Three radioactive compounds were observed on the chromatograms (System A): traces of free serine (R_f 0), phosphatidylserine (R_f 0.18), and, in some experiments, phosphatidylethanolamine (R_f 0.45). The percent of the recovered radioactivity in each peak was determined and the total radioactivity in each phospholipid was calculated by multiplying by the total radioactivity in the lower phase. Boiled protein controls were incubated and any radioactivity present was subtracted in making calculations. In Table 3 and Figure 3, the rate of phosphatidylethanolamine formation was calculated taking into account the lower specific activity of this product (⅔ of that of L-[U-^{14}C]serine). In the other experiments, rates were calculated based only on the specific activity of L-[U-^{14}C]serine. In some experiments, the radioactive lipid extracts were chromatographed on 0.25-mm layers of silica gel H prepared with 1 mM Na_2CO_3 and developed with chloroform–methanol–glacial acetic acid–water 50:25:7:3 (by volume) (System B).

The incorporation of L-[U-^{14}C]serine into phospholipids by microsomes and tumor mitochondria was linear for 10 min and continued at a substantial, nearly linear rate for 20 min. The activity was linear with protein to at least 3 mg/ml. 1.5 mM L-[U-^{14}C]serine was found to be saturating in all preparations and optimal reaction rates were noted in the presence of 10 mM Ca^{2+}. The enzyme catalyzing phosphatidylserine synthesis in normal and tumor membranes lost activity gradually with storage at −60°C, but after 4–6 weeks marked losses of activity were often noted. The experiments reported here were done on membrane preparations that had been stored at −60°C for less than 3 weeks.

The ^{14}C-labeled phosphatidylserine formed in the base-exchange reaction with 7777 mitochondria and microsomes was subjected to alkaline hydrolysis by the method of Dawson (18). In both cases, greater than 96% of the ^{14}C label was recovered in the aqueous phase, indicating that diacyl forms predominate in the respective products. The ^{14}C-labeled phosphatidylserines produced by 7777 mitochondria and microsomes were also subjected to phospholipase D hydrolysis as described by Long, Odavic, and Sargent (19). After incubations for 48 hr at 20°C, the mixture was extracted by the method of Folch et al. (17). More than 98% of the radioactivity was found in the aqueous phase of the extraction; less than 2% of the radioactivity was present in the phosphatidic acid produced by phospholipase D hydrolysis of the respective ^{14}C-labeled phosphatidylserines.

Chemicals

L-[U-^{14}C]Serine was obtained from Amersham Corporation, Arlington Heights, IL. Unlabeled serine and NADPH were obtained from Sigma, St. Louis, MO. Cytochrome *c* was obtained from EM Reagents, Elmsford, NY. Phospholipase D (cabbage) was purchased from Calbiochem, La Jolla, CA. Other chemicals were of analytic reagent grade quality. Chloroform and methanol were redistilled before use.

RESULTS

Table 1 shows the rate of incorporation of L-[U-^{14}C]serine into phospholipids at saturating concentrations of substrate in the presence of 10 mM Ca^{2+}. In all tissues studied the rate of this reaction was most rapid in microsomes, 4.61–5.33 nmol $mg^{-1}hr^{-1}$, and there was no apparent difference between microsomes from normal liver, 7777 hepatoma, fetal liver, or regenerating liver. Mitochondria prepared from the 7777 hepatoma exhibited substantial activity in this reaction, 2.70 nmol mg^{-1} hr^{-1}, while very little activity was found in mitochondria from normal rat liver, fetal rat liver, and regenerating rat liver. The activity in mitochondria from fetal liver and regenerating liver could be accounted for by the contamination with microsomes as measured by rotenone-insensitive NADPH cytochrome *c* reductase. However, the activity of normal rat liver mitochondria exceeded that of the microsomal contamination (relative sp act 12.9 vs. 3.6). Further experiments were carried out to assess the role of microsomal contamination in the observed activity of mitochondrial phosphatidylserine formation from radioactive L-serine.

Fig. 1 shows the results of an experiment where fresh preparations of mitochondria (1 mg) from normal rat liver and the 7777 hepatoma were incubated with increasing amounts of added microsomal protein from normal rat liver or hepatoma, respectively. The position of the lowest point (with regard to microsomal protein) was determined based on the microsomal marker enzyme, rotenone-insensitive NADPH cytochrome *c* reductase (16). Reaction velocity was plotted on the *y*-axis. Regression lines were determined by the method of least squares and the extrapolated *y*-intercept represents the activity with no microsomes present. The results were as follows: normal liver mitochondria, *y*-intercept 0.24,

TABLE 1. Incorporation of L-[U-^{14}C]serine into phospholipid in subcellular fractions of normal rat liver, 7777 hepatoma, fetal and regenerating rat liver, in vitro[a]

	Phospholipid formed nmol mg^{-1} hr^{-1}	R.S.A.[b]	NADPH-cyst *c* Rotenone-insens. nmol mg^{-1} hr^{-1}	R.S.A.
Normal liver				
mitochondria	0.63 ± 0.15(3)	12.9	1.84 ± 1.40(3)	3.6
microsomes	4.86 ± 0.28(4)	100.0	51.30 ± 4.50(3)	100.0
7777 hepatoma				
mitochondria	2.70 ± 0.64(6)[c]	58.6	1.91 ± 0.55(3)	9.0
microsomes	4.61 ± 0.46(3)	100.0	21.20 ± 2.50(3)[c]	100.0
Fetal liver				
mitochondria	0.02 ± 0.03(3)[c]	0.4	1.12 ± 1.08(3)	15.5
microsomes	4.77 ± 1.70(3)	100.0	7.20 ± 3.33(3)[c]	100.0
Regenerating liver				
mitochondria	0.22	4.1	1.08	3.9
microsomes	5.33	100.0	27.60	100.0

[a] Mean ± 1 SD of duplicate determinations. Numbers in parentheses represent the number of preparations studied.

[b] R.S.A., relative specific activity where the most active fraction has been set to 100.

[c] $P < 0.001$ vs. normal liver.

The rates have been calculated from the specific activity of L[U-^{14}C]serine; no correction has been made for the probable presence of [^{14}C]phosphatidylethanolamine in the products of the reaction with 7777 hepatoma mitochondria. The contamination of microsomes with mitochondria was assessed with succinate dehydrogenase; the results were as follows: normal liver, 4.6%; 7777 hepatoma, 3.9%; fetal liver, 0.5%; regenerating liver, 3.8%.

slope 6.78, $r = 0.98$; 7777 hepatoma mitochondria, y-intercept 2.45, slope 4.93, $r = 0.80$. Thus, incorporation of L-[U-^{14}C]serine into phospholipid by base-exchange is virtually absent in normal liver mitochondria (0.24 nmol mg^{-1} hr^{-1}). However, the 7777 mitochondria have substantial activity (2.45 nmol mg^{-1} hr^{-1}) which cannot be explained by microsomal contamination.

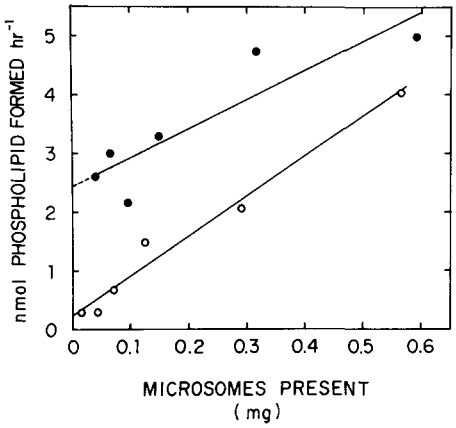

Fig. 1. Incorporation of [^{14}C]serine into phospholipids catalyzed by mitochondria from normal liver or 7777 hepatoma: the effect of added microsomes. Mitochondrial protein (1.0 mg) was incubated with 1.5 mM L-[U-^{14}C]serine and 10 mM $CaCl_2$ for 20 min at 37°C as noted in Methods. In other incubations, different amounts of the respective microsomal proteins were added to 1.0 mg of mitochondrial protein, the reactions rates were determined based on the specific activity of L-[U-^{14}C]serine as noted in Methods and plotted on the ordinate. The positions of the points without added microsomal protein were determined by estimating the degree of microsomal contamination of the respective mitochondrial preparations using rotenone-insensitive NADPH-cytochrome *c* reductase (16). The total amount of microsomal protein in the incubation has been plotted on the abscissa. Regression lines were determined by the method of least squares, and the y-intercepts represent the estimated residual mitochondrial activity without microsomes. Open circles, normal liver mitochondria and added microsomes; closed circles, 7777 hepatoma mitochondria and added microsomes.

Table 2 shows some properties of the incorporation of radioactive L-serine into phospholipid in 7777 hepatoma mitochondria compared with that of normal liver microsomes. Both require Ca^{2+}; this cation cannot be replaced by Mg^{2+}, Mn^{2+}, or Co^{2+}. The reaction is strongly inhibited by detergents and by a sulfhydryl inhibitor. Heating to 70°C for 10 min inhibits the incorporation of L-[U-^{14}C]serine by 96–99%.

The products of the reaction were analyzed by several one-dimensional thin-layer chromatography systems and by two-dimensional thin-layer chromatography. **Fig. 2** shows radioautograms of the lipids extracted from incubations of 7777 mitochondria and normal liver microsomes with L-[U-^{14}C]serine in the presence of 10 mM Ca^{2+}. The adjacent panels show the lipids of normal liver mitochondria and microsomes visualized by treatment with iodine vapors. In normal microsomes, the sole labeled lipid cochromatographed with phosphatidylserine; similar results were obtained with 7777 microsomes (not shown). However, in the 7777 hepatoma, significant amounts of both phosphatidylserine and its decarboxylation product, phosphatidylethanolamine, were seen. Radioactive phosphatidylserine and phosphatidylethanolamine from the incubations also comigrated with authentic reference compounds in

several one-dimensional thin-layer chromatography systems: the R_f values for phosphatidylserine were 0.17 (System A) and 0.63 (System B); the R_f values for phosphatidylethanolamine were 0.49 (A) and 0.78 (B).

Fig. 3 shows the effect of incubation time on the incorporation of L-[U-^{14}C]serine into phosphatidylserine and phosphatidylethanolamine. At 3 min, the earliest time studied, phosphatidylserine formation exceeded that of phosphatidylethanolamine, 78 vs. 45 pmol mg^{-1}. However, phosphatidylserine did not accumulate and plateaued at 100–150 pmol mg^{-1} at 60 min. With microsomes from either normal liver or 7777 hepatoma, only phosphatidylserine was formed (data not shown).

Table 3 shows the effect of potassium cyanide on the rate of phosphatidylserine (and phosphatidylethanolamine) formation by normal liver microsomes and 7777 hepatoma mitochondria; 0.75 mM or 1.0 mM KCN had little effect on phosphatidylserine formation by microsomes. Slight inhibition of total phospholipid formation was found in 7777 mitochondria. However, product analysis by thin-layer chromatography in System A showed that KCN caused a striking reduction in the conversion of phosphatidylserine to phosphatidylethanolamine by 7777 mitochondria. In the absence of KCN, radioactive phosphatidylserine accounted for only 35 or 41% of the total products vs. 92–95% in the presence of 0.75 or 1.0 mM KCN.

DISCUSSION

These studies demonstrate the presence in 7777 hepatoma mitochondria of the enzyme catalyzing the incorporation of L-serine into phosphatidylserine. This enzyme activity is essentially absent in mitochondria from rat liver, in agreement with the results obtained by previous workers (4, 6). When we examined mitochondria from the rapid-growth controls, fetal liver and regenerating liver, no evidence for L-serine incorporation by base-exchange could be found (Table 1), suggesting that malignant transformation is responsible for this apparent aberration in mitochondrial biogenesis in the 7777 hepatoma.

Of interest is the observation (Fig. 3) that phosphatidylserine formed by 7777 hepatoma mitochondria does not accumulate but is decarboxylated to phosphatidylethanolamine which accumulates with increasing incubation time. The presence of Ca^{2+}-dependent base-exchange and phosphatidylserine decarboxylase in 7777 hepatoma mitochondria is analagous to the results reported by Dennis and Kennedy for *Tetrahymena pyriformis* (20).

TABLE 2. Properties of L-[U-^{14}C]serine incorporation into phospholipids of 7777 hepatoma mitochondria and normal liver microsomes, in vitro

Conditions	7777 Hepatoma Mitochondria	Normal Liver Microsomes
Complete	100.0	100.0
Ca^{2+} omitted	0.3	4.1
5 mM Mg^{2+}	5.7	3.4
5 mM Mn^{2+}	0.0	10.7
5 mM Co^{2+}	2.4	0.5
10 mM Ca^{2+}; 5 mM EDTA	4.9	5.5
1 mM *p*-chloromercuriobenzoate	10.6	9.4
0.5 mg/ml deoxycholate	2.3	0.0
0.5 mg/ml Triton X-100	4.3	6.0
70°C × 10 min	6.5	0.9

The incubations contained 50 mM Tris-HCl, pH 7.4, 1.5 mM L-[U-^{14}C]serine, sp act 4.63 mCi/mmol; 1.5 mg/ml protein; and 10 mM $CaCl_2$. Alterations in the reaction mixture were made as noted above. The respective mixtures were incubated at 37°C for 20 min and the incorporation of [^{14}C]serine into total phospholipid was determined as noted in Methods. The results have been expressed as percent of the control. Control reaction rates were: 7777 hepatoma mitochondria, 2.68 nmol mg^{-1} hr^{-1}; normal liver microsomes, 3.83 nmol mg^{-1} hr^{-1}, based on the specific activity of L-[U-^{14}C]serine as in Table 1.

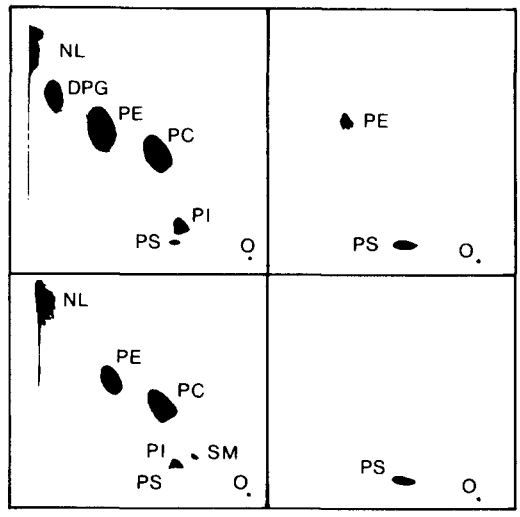

Fig. 2. Two-dimensional thin-layer chromatography and radioautography of the reaction products of 7777 hepatoma mitochondrial and normal liver microsomes. Normal liver microsomes and 7777 hepatoma mitochondria were incubated with L-[U-^{14}C]-serine as noted in Methods and the washed lipid extracts were chromatographed on 0.25-mm layers of silica gel H containing 5.5% magnesium acetate (w/w) as previously described (9). The chromatograms were developed in the first dimension with chloroform–methanol–conc. ammonia–water 60:30:2:1.5, by volume. After drying in a nitrogen atmosphere for 30 min the plates were developed in the second dimension with chloroform–acetone–methanol–glacial acetic acid–water 3:4:1:1:0.5, by volume (9). Lipid spots were visualized with iodine vapors (left panels) or by radioautography with Kodak No-Screen X-ray film (right panels). Thin-layer plates and radioautograms were photographed with a Polaroid MP3 camera using Type 51 film. Upper right, radioautogram of the lipid extract of 7777 hepatoma mitochondria. Lower right, radioautogram of lipid products of normal liver microsomes. Upper left, lipids of normal liver mitochondria. Lower left, lipids of normal liver microsomes, visualized by iodine vapors.

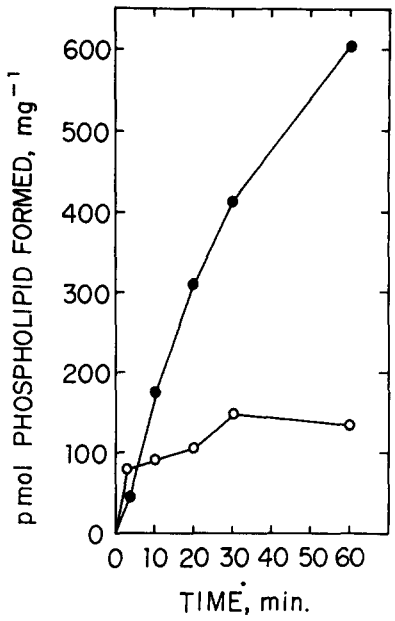

Fig. 3. Time course of L-[U-^{14}C]serine incorporation into phosphatidylserine and phosphatidylethanolamine catalyzed by 7777 hepatoma mitochondria. Incubations were carried out at 37°C as noted in Methods and analyzed by thin-layer chromatography in system A at the times indicated on the abscissa. The results are expressed as picomol product mg^{-1}. The rate of phosphatidylethanolamine formation has been calculated using the correction noted in Methods. Open circles, phosphatidylserine; closed circles, phosphatidylethanolamine.

Phosphatidylserine decarboxylase in 7777 hepatoma mitochondria was inhibited by 1 mM KCN (Table 3). To our knowledge, this is the first report of KCN inhibition of phosphatidylserine decarboxylase. The mechanism of the inhibition is presently obscure. Satre and Kennedy (21) recently reported that purified phosphatidylserine decarboxylase from *Escherichia coli* contains bound pyruvate; the carbonyl group of this pyruvate residue is essential for its catalytic activity (21). However, KCN has not yet been tested as a potential inhibitor of purified bacterial phosphatidylserine decarboxylase. The mammalian enzyme has not been extensively characterized.

The presence of Ca^{2+}-stimulated base-exchange activity in 7777 hepatoma mitochondria might be expected to lead to an increased synthesis of phosphatidylserine and, by decarboxylation, of phosphatidylethanolamine. However, it cannot account for the net increase in mitochondrial total phospholipid relative to protein as previously reported in the 7777 hepatoma (9, 10). On the other hand, alterations in the relative ratios of phosphatidylcholine and phosphatidylethanolamine can be explained by the presence of this pathway in the hepatoma.

This represents the second example of the altered intracellular distribution of a membrane-bound enzyme involved in phospholipid biosynthesis in the 7777 hepatoma. In addition, transglutaminase, an enzyme that is normally cytosolic, has been reported to be principally membrane-bound in the Novikoff hepatoma (22). Our previous studies in the 7777 hepatoma have shown a greatly increased activity of CTP: phosphatidate cytidylyltransferase (EC 2.7.7.41) in mitochondria (12), particularly in the outer mitochondrial membrane in the tumor (11). This enzyme is principally microsomal in normal liver (6, 11). However, other membrane-bound enzymes of de novo phospholipid biosynthesis had a normal subcellular localization in the 7777 hepatoma including *1*) acylCoA: *sn*-glycerol-3-phosphate acyltransferase (EC 2.3.1.5); *2*) CDP-diacylglycerol: *sn*-glycerol-3-phos-

TABLE 3. Analysis of the phospholipid products formed from L-[U-^{14}C]serine by 7777 hepatoma mitochondria and normal liver microsomes: the effect of KCN

Incubation Conditions	PS Formed nmol mg^{-1} hr^{-1}	PE Formed nmol mg^{-1} hr^{-1}	Total Phospholipid nmol mg^{-1} hr^{-1}
Experiment 1			
microsomes	4.01	0.05	4.06
microsomes + 0.75 mM KCN	3.89	0.18	4.07
7777 mitochondria	1.08	1.53	2.61
7777 mito + 0.75 mM KCN	2.15	0.18	2.33
Experiment 2			
microsomes	4.50	0.15	4.65
microsomes + 1 mM KCN	4.73	0.11	4.84
7777 mitochondria	0.80	1.46	2.26
7777 mito + 1 mM KCN	1.64	0.09	1.73

Incubations were done as described in Methods with additions as noted. The washed lipid extracts were counted and analyzed by thin-layer chromatography with System A. Boiled protein controls have been subtracted to calculate the results above, and the rate of PE formation has been calculated using the correction noted in Methods. PS, phosphatidylserine; PE, phosphatidylethanolamine.

phate phosphatidyltransferase (EC 2.7.8.5) and phosphatidylglycerolphosphate phosphatase (EC 3.1.3.37); and *3*) CDP-choline: diacylglycerol cholinephosphotransferase (EC 2.7.8.2) (11). Since the intracellular localization of CTP: phosphatidate cytidylyltransferase and Ca^{2+}-dependent base-exchange was normal in fetal and regenerating rat liver (ref. 11, this publication), it appears that rapid growth alone is not sufficient to explain the findings. The significance of the presence of these two enzymes in hepatoma mitochondria is currently unclear, but they might confer some advantages in the capacity of tumor mitochondria to generate membrane phospholipids during the rapid growth associated with malignant transformation.

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