Substrate Preference in Phosphatidylserine Biosynthesis for Docosahexaenoic Acid Containing Species

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Received July 8, 2003; Revised Manuscript Received November 12, 2003

ABSTRACT: Neuronal membranes contain high levels of phosphatidylserine (PS) and docosahexaenoic acid (22:6n-3, DHA). In this study, substrate preference in PS synthesis was determined to gain insight on the biochemical basis for concentrating PS in neuronal membranes where 22:6n-3 is highly enriched. We first established an in vitro assay method using unilamellar vesicles (LUV) of deuterium-labeled substrates and reversed-phase HPLC/electrospray ionization (ESI) mass spectrometry. The PS production by the incubation of deuterium-labeled substrate and microsomal fractions was monitored. We found that tissue-specific substrate preference exists in PS synthesis. Microsomes from the cerebral cortex synthesized PS from 18:0,22:6-PC most favorably among the PC substrates tested, followed by 18:0,22:5-PC, resulting in the PC substrate preference in the order of 18:0,22:6 > 18:0,22:5 > 18:0,20:4 = 18:0,18:1. Liver microsomes also preferred 18:0,22:6-PC as the substrate in PS synthesis but did not use 18:0,22:5-PC favorably. The 18:0,22:5-PC species was converted to PS at the similar extent as 18:0,20:4- or 18:0,18:1-PC species in the liver. Both brain and liver microsomes showed a preference for 18:0 over 16:0 as the *sn*-1 fatty acid. From these data it was deduced that preferential conversion of 18:0,22:6-PC to the corresponding PS species is at least partly responsible for concentrating PS in neuronal tissues where 22:6n-3 is particularly abundant. The distinctive preference for 18:0,22:5-PS observed with brain microsomes may help to maintain PS at a high level in the brain when 22:6n-3 is replaced by 22:5n-3 as in the case of n-3 fatty acid deficiency.

Phosphatidylserine (PS)[1] constitutes the major anionic phospholipid in many eukaryotic biomembranes. The role of PS in various signaling events such as protein kinase activation, blood coagulation, and recognition and removal of apoptotic cells by macrophages has been well established (*1*−*4*). Neuronal tissues contain particularly high levels of PS, in comparison to other tissues (*5*, *6*), although the biochemical mechanism for this enrichment is not well established. Neuronal membranes are also enriched with docosahexaenoic acid (22:6n-3, DHA) (*7*, *8*). Among individual brain phospholipid classes, PS contains the highest proportion of 22:6n-3 comprising nearly 35% of the total fatty acyl composition in PS (*9*).

During n-3 fatty acid deficiency, 22:6n-3 composition decreases significantly, and docosapentaenoic acid (22:5n-6, DPA), an n-6 fatty acid counterpart of 22:6n-3, replaces 22:6n-3 (*10*). Long-term ethanol exposure can also decrease the 22:6n-3 content in the brain (*11*, *12*). We have previously reported that depletion of 22:6n-3 by n-3 fatty acid deficiency (*13*−*15*) or long-term ethanol exposure (*16*) decreases PS content selectively in neuronal membranes. Consistently, PS biosynthetic activity as well as 18:0,22:6-PS accumulation has been shown to be hampered by n-3 fatty acid deficiency or ethanol treatment (*13*, *16*). On the contrary, the PS content is increased with enrichment of neuronal or glial cells with 22:6n-3, due to accumulation of 18:0,22:6-PS (*13*, *16*−*18*). These findings consistently suggest that 22:6n-3 positively modulates PS accumulation in the brain.

In animal cells PS is synthesized from preexisting phosphatidylcholine (PC) or phosphatidylethanolamine (PE) by exchanging choline or ethanolamine with L-serine. This reaction occurs in the endoplasmic reticulum and mitochondria-associated membranes (MAM) catalyzed by phosphatidylserine synthase I (PSS1) or phosphatidylserine synthase II (PSS2), each of which uses PC or PE as the primary substrate (*19*−*22*). The PS molecules can be transported from its site of synthesis to the mitochondria and converted to PE by PS decarboxylation (PSD) (*23*). Therefore, distinctive accumulation of 22:6n-3-containing PS species in the brain may involve the substrate specificity in any of these processes, for example, favored PS biosynthesis or nonfavored degradation through PS decarboxylation for 22:6n-3-containing molecular species. Upon examination of the substrate specificity in PS decarboxylation, we found that the 22:6n-3 species is the most favored substrate for

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[1] Abbreviations: 22:6n-3 (DHA), docosahexaenoic acid; PS, phosphatidylserine; 22:5n-6, docosapentaenoic acid; 20:5n-6, eicosapentaenoic acid; PC, phosphatidylcholoine; HPLC/ESI MS, high-performance liquid chromatography/electrospray ionization mass spectrometry; 18:0,22:6, 1-stearoyl-2-docosahexanoyl; 18:0,22:5, 1-stearoyl-2-docosapentanoyl; 18:0,18:1, 1-stearoyl-2-oleyl; 18:0,20:4, 1-stearoyl-2-arachidonyl; 16:0,16:0 (di16:0), 1-palmitoyl-2-palmitoyl; 18:0,18:0 (di18:0), 1-stearoyl-2-stearoyl; PSS, phosphatidylserine synthase; LUV, unilamellar vesicles; DTPA, diethylenetriaminepentaacetic acid.

10.1021/bi035197x
Published on Web 01/06/2004

mitochondrial PSD (*24*), suggesting that pathways other than PSD contribute to the accumulation of 22:6n-3 in PS. To understand biochemical mechanisms of concentrating PS in neuronal membranes where the 22:6n-3 level is high, we investigated substrate preference in PS biosynthesis in vitro. By incubating microsomes with deuterium-labeled PC molecular species in unilamellar vesicles and monitoring product formation by reversed-phase HPLC/electrospray mass spectrometry, we found that 18:0,22:6-PC is particularly preferred in the brain PS biosynthesis, supporting the positive role of 22:6n-3 in accumulating PS in neuronal membranes.

MATERIALS AND METHODS

Deuterium-labeled phospholipid standards were purchased from or custom synthesized by Avanti Polar Lipids (Alabaster, AL). Docosapentaenoic acid (22:5n-6) used for phospholipid synthesis was a generous gift from Omegatech (currently incorporated into Martek, Columbia, MD) and was urea-crystallized by Nu-Check (Elysian, MN). All solvents were of HPLC grade and were purchased from EM Scientific or Burdick & Jackson (Muskegon, MI). BF_3/methanol was obtained from Altech (Deerfield, IL). Deuterium-labeled phospholipids used for liposome preparation were calibrated by phosphorus assay and GC analysis as described earlier (*14*).

Isolation of Microsomal Fraction. Brain and liver microsomal fractions were prepared according to Cotman with slight modifications (*25*). Homogenates of the tissues were prepared in a solution of 0.32 M sucrose with 10 mM Tris-HCl and 1 mM EDTA (pH 7.3) and centrifuged at 1000*g* for 5 min to remove nuclei and unbroken cells. The resulting supernatant was transferred and centrifuged at 12500*g* for 12 min. The supernatant from this spin was collected and spun for 60 min at 100000*g*. The microsomal fraction, which precipitated at the bottom, was washed with phosphate-buffered saline (PBS) and reconstituted in 0.25 M sucrose containing 3 mM HEPES (pH 7.5). A protein assay was performed using the bicinchoninic acid reagent (*26*), and lipids were extracted according to the method of Bligh and Dyer (*27*).

Preparation of Unilamellar Vesicles. Unilamellar vesicles (LUV) of deuterium-labeled PC were prepared by the following method. Equimolar amounts of d_{35}-18:0,22:6-PC, d_{35}-18:0,22:5-PC, d_{35}-18:0,18:1-PC, and d_{35}-18:0,20:4-PC were dissolved in chloroform with 16:0,18:1-PA in the ratio of 3:2 PC:PA, and the mixture was dried under argon. 16:0,18:1-PA was included to promote the fusion of the liposomes to microsomes as reported earlier (*28*). The lipids were reconstituted in 2 mL of 75 μM 2,6-di-*tert*-butyl-*p*-cresol (BHT) in cyclohexane. Samples were lyophilized under vacuum and purged under argon before the samples were reconstituted in 0.25 M sucrose solution containing 3 mM HEPES (pH 7.5) and 50 μM diethylenetriaminepentaacetic acid (DPTA) in an argon box. Solutions were vortexed until a colloidal suspension was formed and then passed through a 0.1 μm polycarbonate filter on a mini-extruder (Avanti Polar Lipids) 10 times to make unilamellar vesicles.

Incubation of Unilamellar Vesicles with Microsomal Fractions. Microsomal fractions of rat brain cortex and liver were incubated with the prepared unilamellar vesicles in 0.25 M sucrose solution containing 3 mM HEPES (pH 7.5), 6 mM $CaCl_2$, and 0−6 mM serine at 37 °C in a shaking water bath. The total volume was kept at 1.8 mL. The reaction was stopped by adding a cold mixture of $CHCl_3$/CH_3OH/CH_3OH−BHT (1:1:1), and the lipids were extracted according to the Bligh and Dyer method in the presence of d_0- or d_{62}-16:0,16:0 PS and d_{35}-18:0,18:0 PC as internal standards (*14*, *15*, *29*).

Phospholipid Molecular Species Analysis. Phospholipids were determined using reversed-phase HPLC/electrospray ionization mass spectrometry as described previously (*14*−*16*, *24*, *29*). Individual molecular species were separated using a C18 column (Prodigy, 150 × 2.0 mm, 5 μm; Phenomenex, Torrance, CA) with a mobile phase containing water:0.5% ammonium hydroxide in methanol:hexane, changing from 12:88:0 to 0:88:12 in 17 min after the initial composition was held for 3 min at a flow rate of 0.5 mL/min. An Agilent Series 1100 MSD instrument, equipped with an ESI source, was used to detect the separated molecular species. The drying gas temperature was 350 °C while the drying gas flow rate and nebulizing gas pressure were 13 L/min and 50 psi, respectively. The capillary and the exit voltages were set at 4000 and 200 V, respectively. Since the instrumental responses are sensitive to the nature of the phospholipid headgroup (*14*, *29*), quantitation was based on the area ratio calculated against the internal standard of the same phospholipid class. The variation of the response for different molecular species within a class was no more than 10%.

RESULTS

When unilamellar vesicles (LUV) containing various deuterium-labeled PC species were incubated with the microsomal fraction, the formation of deuterium-labeled 18:0,22:6-, 18:0,22:5-, 18:0,18:1-, and 18:0,20:4-PS from the corresponding PC was observed. As an example, representative mass chromatograms are shown in Figure 1 for the PS production from deuterium-labeled PC substrates by brain cortex microsomes. Each PS molecular species was detected as diglyceride ions while substrate PC species were detected as $[M + H]^+$ ions as has been reported earlier (*17*, *29*). Substrate specificity in PS biosynthesis was determined by quantifying produced PS molecular species against the PS internal standard. As the PS internal standard, di16:0-PS rather than deuterium-labeled di-16:0-PS was used in this particular example since this species was not present in the brain microsomes at a measurable level.

When serine was not added in the incubation mixture exogenously, the PS synthesis by brain microsomes was trivial. Among the PC substrates tested, conversion to the corresponding PS species was detected only for 18:0,22:6-PC during the entire incubation period of 135 min using a protein amount of up to 750 μg (Figure 2). The PS synthesis in this case presumably relied strictly on the presence of endogenous serine in microsomes. Similarly, liver microsomes converted only 18:0,22:6-PC although the PS production was slightly lower (data not shown).

Addition of serine in the incubation mixture increased the PS production considerably from all four PC molecular species in the liposomes (Figure 3). However, the 18:0,22:6 species still remained to be the best substrate for PS synthesis

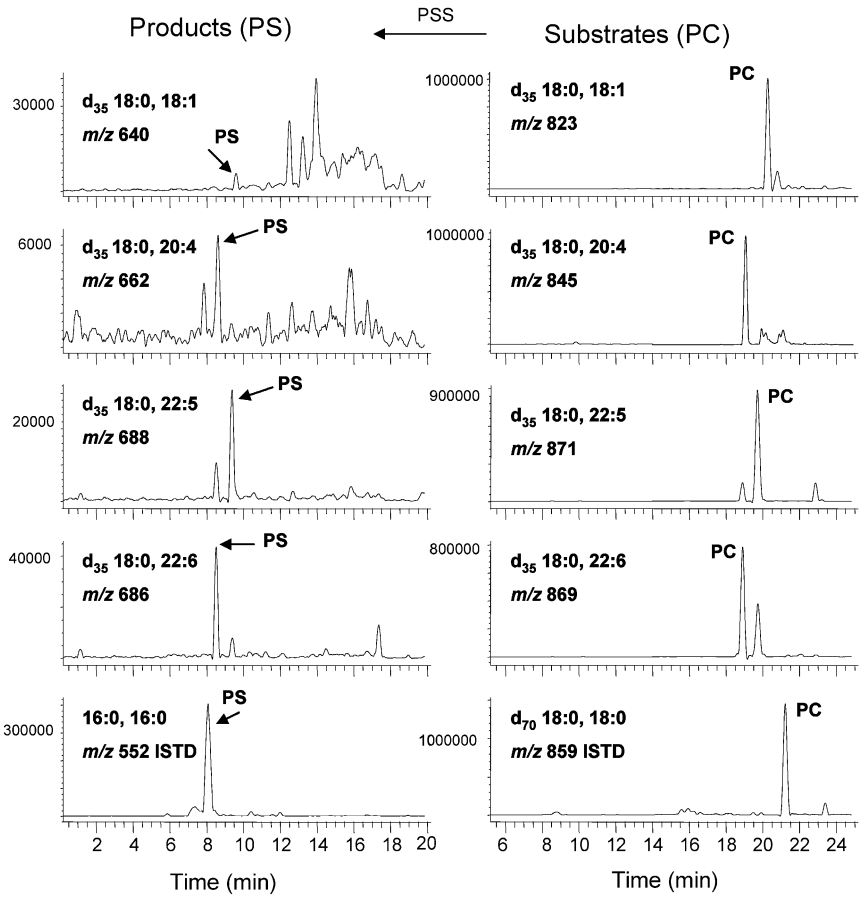

FIGURE 1: Representative mass chromatograms monitoring PS synthesis from PC substrates. Liposomes containing 50 μM each of deuterium-labeled PC substrates were incubated with 750 μg of brain microsomal protein for 135 min in the presence of 1.5 mM serine, and the PS product formation was monitored by reversed-phase HPLC/ESI-MS. Di16:0-PS and d_{35}-di18:0-PC were used as internal standards for quantitation for PS and PC species, respectively.

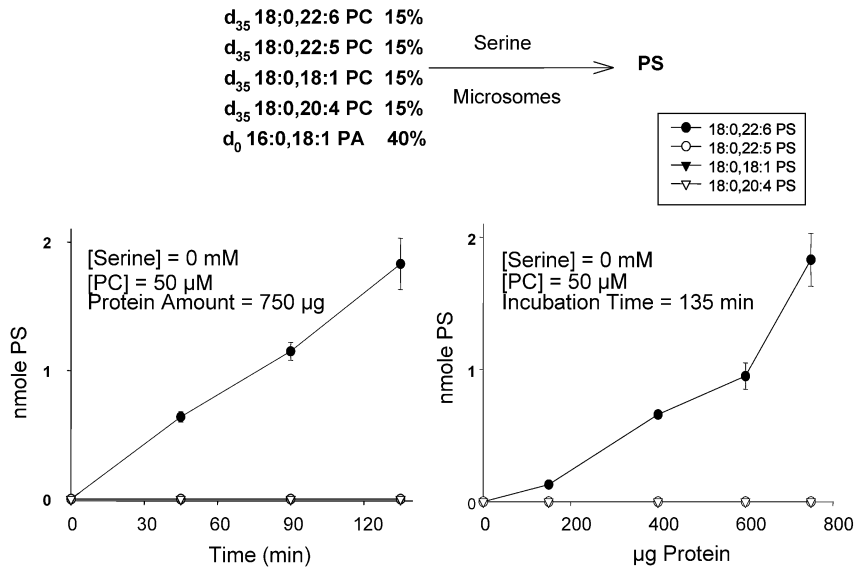

FIGURE 2: PS production as a function of time and protein amount without exogenously added serine. Brain cortex microsomes corresponding to 0−750 μg of protein were incubated with liposomes containing 50 μM each of deuterium-labeled PC substrates for indicated time periods. PS production was detected only for the 18:0,22:6 species.

by brain or liver microsomes for all of the serine concentrations tested. PS biosynthetic activity of brain cortex microsomes was in the order of 18:0,22:6 > 18:0,22:5 > 18:0,20:4 ≥ 18:0,18:1-PS. Liver microsomes showed the substrate preference in the order of 18:0,22:6 > 18:0,20:4 ≥ 18:0,22:5 > 18:0,18:1 species. When liposomes containing 50 μM each of PC species were incubated with 750 μg of microsomal protein for 135 min, the maximum conversion by brain and liver microsomes was similar for 18:0,22:6 or 18:0,18:1 species. The production of 18:0,22:5-PS exhibited distinctive tissue specificity. Only brain microsomes were capable of synthesizing 18:0,22:5-PS at a significantly higher

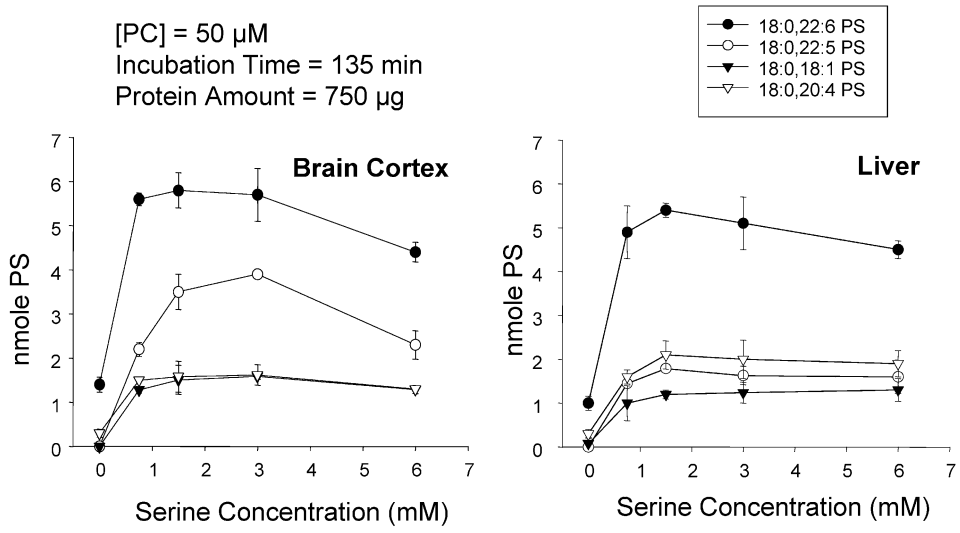

FIGURE 3: Effect of serine concentration on PS production. Liposomes containing 50 μM each of deuterium-labeled PC substrates were incubated with 750 μg of brain cortex or liver microsomal proteins for 135 min.

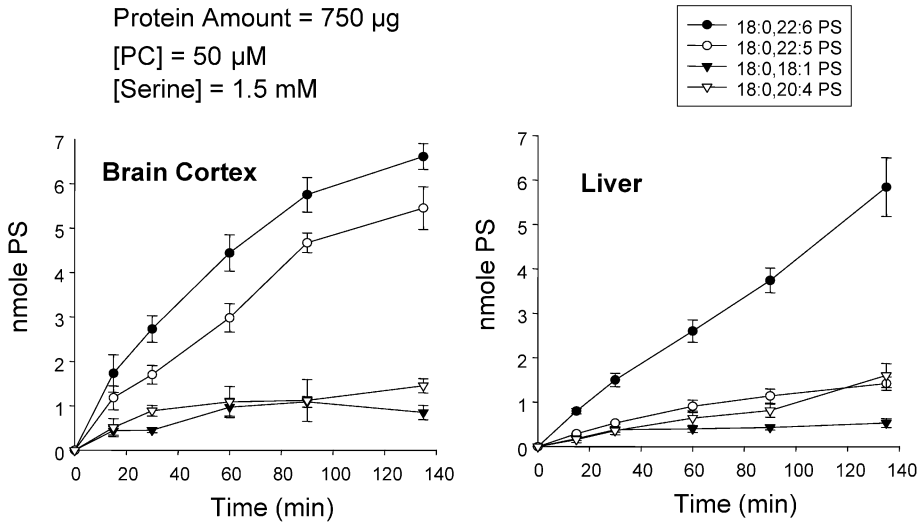

FIGURE 4: Time course of PS production from liposomes containing 50 μM each of deuterium-labeled PC substrates incubated with 750 μg of brain cortex or liver microsomes in the presence of 1.5 mM serine.

level in comparison to 18:0,20:4 or 18:0,18:1 species, and this conversion required the presence of serine in the medium. Without exogenously added serine, PS synthesis by brain microsomes from 18:0,22:5-PC was also negligible as indicated in Figure 2. The optimum conversion was observed with microsomes from both tissues at the serine concentration of 0.75−1.5 mM for all of the molecular species tested, with the exception of 18:0,22:5-PS. The synthesis of this species by brain cortex microsomes was optimized at a serine concentration between 1.5 and 3 mM. The serine concentration above 3 mM appeared to be inhibitory.

Figure 4 presents the time course of the PS production from 50 μM each of PC molecular species by 750 μg of brain cortex or liver microsomes in the presence of 1.5 mM serine. The time-dependent production of 22:6- and 18:0,22:5-PS species was observed for brain microsomes, despite a slight decrease in the rate of production after 90 min. In contrast, the increase of 18:0,20:4 or 18:0,18:1 appeared to be minimal. Likewise, liver microsomes produced 18:0,22:6-PS in a time-dependent manner, although the initial rate of conversion (approximately 50 pmol/min) was significantly less than that observed with brain microsomes (approximately 100 pmol/min). The production of 18:0,22:5-PS was as slow as 18:0,20:4-PS, followed by 18:0,18:1-PS which did not show any further increase after 30 min incubation.

The observed characteristic molecular species specificity in PS synthesis was maintained in a wide range of substrate concentrations as shown in Figure 5. The production of 18:0,22:6- and 18:0,22:5-PS showed a substrate concentration-dependent increase, which gradually leveled off at a PC concentration above 50 μM. A similar increase for 18:0,22:6-PS was also seen with liver microsomes. In agreement with the data presented in Figures 2−4, no significant production of 18:0,22:5-PS by liver microsomes was observed at all PC concentrations tested. Likewise, the increase in PC concentration appeared to have little effect on the synthesis of 18:0,20:4- and 18:0,18:1-PS for both brain and liver microsomes.

The effect of the *sn*-1 fatty acyl chain on the PS synthetic activity was examined using the PC species with 18:0 or 16:0 at the *sn*-1 position. Both brain and liver microsomes favored PC molecular species with 18:0 at the *sn*-1 position

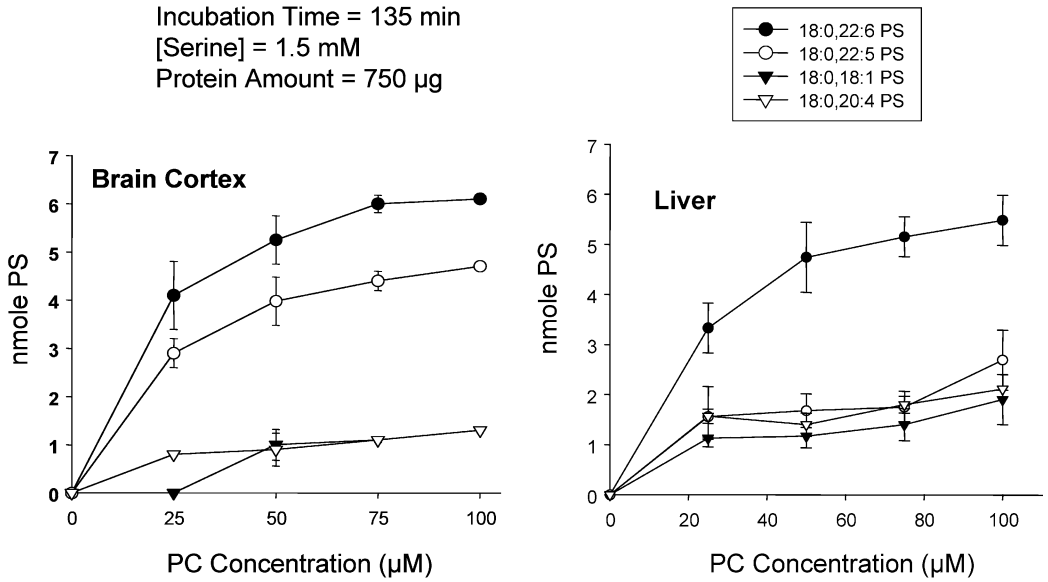

FIGURE 5: Effect of PC substrate concentration on PS production. Liposomes containing various concentrations of deuterium-labeled PC substrates were incubated with 750 μg of brain cortex or liver microsomal proteins for 135 min in the presence of 1.5 mM serine. The concentration of each deuterium-labeled PC species is indicated in the figure.

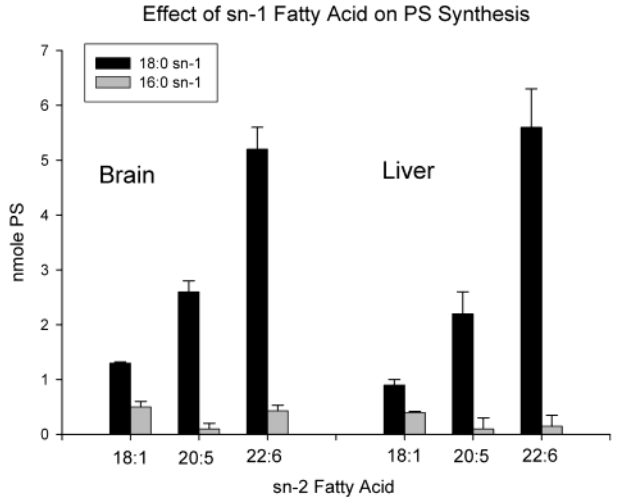

FIGURE 6: Effect of *sn*-1 fatty acid on PS synthesis. Liposomes containing 50 μM each of PC substrates were incubated with 750 μg of brain cortex or liver microsomal proteins for 135 min.

in comparison to their 16:0 counterpart at least for the three pairs of PC species examined, which contain 18:1, 20:5, or 22:6 at the *sn*-2 position (Figure 6). For all of these pairs, PS synthesis from the PC containing 16:0 at the *sn*-1 position was negligible in comparison to 18:0 PC species.

The data obtained in our system used deuterium-labeled exogenous PC substrates to monitor PS biosynthesis in the presence of the endogenous microsomal phospholipids. The endogenous PC and PS molecular species profile in brain microsomes was not altered due to the 135 min incubation with 50 μM exogenous PC substrate (Table 1), indicating that no competition occurred between endogenous and exogenously added PC species during the incubation period. The substrate preference observed in this study consistently indicated that brain PS synthesis from PC was favored in the order of 18:0,22:6 > 18:0,22:5 >18:0,20:4 ≥ 18:0,18:1-PS, while in the liver the PS production was in the order of 18:0,22:6 ≫ 18:0,20:4 ≥ 18:0,22:5 > 18:0,18:1. In an attempt to relate this biosynthetic substrate preference to the phospholipid profile, the microsomal PS

Table 1: Effect of Exogenous Substrates on Endogenous Levels of Brain Microsomal PS or PC[a]

	0 min incubation		135 min incubation	
	PS	PC	PS	PC
18:0,18:1	0.6 ± 0.1	9.6 ± 1.1	0.6 ± 0.1	9.3 ± 1.2
18:0,20:4	0.2 ± 0.0	4.6 ± 0.1	0.2 ± 0.0	4.9 ± 0.5
18:0,22:6	4.5 ± 0.2	0.6 ± 0.1	4.3 ± 0.2	0.5 ± 0.1
18:0,22:5	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1

[a] Data expressed as the mean ± SD of the d_0/d_{35} ratio (n = 3) corresponding to the (peak area of endogenous PS or PC molecular species)/(peak area of the deuterium-labeled PS or PC internal standard).

Table 2: Microsomal PS and PC Profile in Brain Cortex and Liver[a]

molecular species	brain cortex		liver	
	PS	PC	PS	PC
18:0,18:1	8.8 ± 0.8	17.8 ± 1.1	1.8 ± 0.1	6.1 ± 0.8
18:0,20:4	2.6 ± 0.1	8.7 ± 0.7	45.2 ± 0.1	17.2 ± 0.3
18:0,22:6	63.4 ± 2.3	1.1 ± 0.1	37.1 ± 0.8	2.6 ± 0.2
18:0,22:5	5.9 ± 0.6	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.1

[a] Data are expressed as the mean ± SD of mol % distribution (n = 3).

composition was analyzed (Table 2). Brain cortex microsomes contained 18:0,22:6-PS as the most abundant PS species (63.4%), which is consistent with the substrate preference observed in this study. The brain microsomes contained 18:0,18:1- (8.8%), 18:0,22:5- (5.9%), or 18:0,20:4-PS (2.6%) at a far less content than 18:0,22:6-PS. Considering the fact that the brain normally contains 22:5n-6 at a level less than 3% of the total fatty acid, the low level of 18:0,22:5-PS is not unexpected. This was also indicated by the low 18:0,22:5-PC pool (0.6%) in brain microsomes. The liver PS profile, however, showed 18:0,20:4-PS as the major species (45.2%) followed by 18:0,22:6 > 18:0,18:1 > 18:0,22:5, despite the distinctive preference for the synthesis of 18:0,22:6-PS. The PC profile indicated that brain and liver microsomes contain abundant 18:0,18:1 and 18:0,20:4 species, respectively. Since availability of substrates, in addition to the PS biosynthetic activity, can also contribute to the

phospholipid profile, it is suggested that the high level of 18:0,18:1- and 18:0,20:4-PS observed in the brain and liver microsomes may be due to the high level of these substrates.

DISCUSSION

In neuronal membranes, 18:0,22:6 is the major PS molecular species, often comprising more than 60% of the total PS composition. Concentration of this species in PS may be due to preferential biosynthesis or nonfavored degradation. Mitochondrial PS decarboxylation, however, uses 18:0,22:6-PS as the best substrate (*24*), indicating that preferred PS synthesis may be the key to the observed accumulation in 18:0,22:6-PS. In this study, we provide evidence that substrate preference indeed exists for this species in PS biosynthesis. To evaluate PS biosynthetic activity, we first established an in vitro assay method using liposomes containing deuterium-labeled PC and reversed-phase HPLC/electrospray ionization mass spectrometry. This approach allowed the direct determination of substrate specificity without labeling, laborious HPLC or TLC separation of labeled PS species, and assignment based on the retention behavior or gas chromatographic analysis of collected fractions (*30*). The synthesis of PS from deuterium-labeled substrates can be easily distinguished from endogenous phospholipids. Quantitative information can be simultaneously obtained for both endogenous and exogenous phospholipid molecular species using internal standards, providing a rapid and convenient method for assaying PS biosynthetic activity.

Both brain and liver microsomal preparations showed a similar substrate preference for PS synthesis in that 18:0,22:6-PC was the favorite substrate and 18:0,18:1- or 18:0,20:4-PC was not favored. A distinctive tissue specificity observed for the synthesis of 18:0,22:5-PS is noteworthy. Usually 22:5n-6 is a minor fatty acid present in brain tissues but accumulates under an n-3 fatty acid deficient condition at the expense of 22:6n-3 (*10*). Only brain microsomes converted 18:0,22:5-PC to the corresponding PS species at a significant extent, corroborating with the previous finding that substantial increases of 18:0,22:5-PS occur only in neuronal tissues under an n-3 fatty acid deficiency condition (*14*).

In neuronal membranes, 18:0,22:6-PC is normally found at a low level. However, under an n-3 fatty acid deficient condition substantial accumulation of 18:0,22:5-PC was observed with concomitant reduction of the PS level (*13*−*15*). This is consistent with the present finding that 18:0,22:5-PC is the less preferred substrate for PS synthesis in comparison to 18:0,22:6-PC. Although association of n-3 fatty acid deficiency with deficits in neuronal function such as learning and memory has been well documented (*31*−*34*), specific roles played by 18:0,22:5-PC in such states have not been established.

Since the study is aimed to determine the tissue-specific substrate specificity, all of the substrates were mixed in the unilamellar vesicles and presented to microsomes at the same time. Although the true enzyme kinetics may be difficult to evaluate under such conditions, the apparent V_{max} and K_m values were derived from the data set shown in Figure 5 for brain microsomal PS synthesis. The apparent V_{max} values were 75.7 and 59.1 pmol mg^{-1} min^{-1} for 18:0,22:6- and 18:0,22:5-PS synthesis, respectively, indicating that 18:0,22:6 turnover was higher than 18:0,22:5. The apparent K_m of 26.4 μM for 18:0,22:5-PC in comparison to 21.7 μM for 18:0,22:6 also corroborated the less favored conversion of 22:5 species in comparison to 22:6 species.

The fact that both brain and liver microsomes favored PC species containing 18:0 over 16:0 at the *sn*-1 position is consistent with the PS profiles in both liver and brain, which are dominated with species containing 18:0 at the *sn*-1 position. However, the preferred PS synthesis for 18:0,22:6 species shown by brain and liver microsomes was not reflected by the endogenous PS profile in the liver (Table 2). Since tissue phospholipid profiles are dynamically maintained via various biosynthetic, metabolic, and remodeling processes, substrate preference in any of these pathways can influence the liver microsomal PS profile. We have previously shown that PS decarboxylation in the liver uses 18:0,22:6-PS as the best substrate (*24*). It is possible that the brain and the liver may have different capability of transporting newly synthesized PS to mitochondria for decarboxylation, so that 18:0,22:6-PS is readily degraded in the liver but maintained in brain. Alternatively, PSS2, which uses PE as the substrate, may contribute to the PS profile in liver microsomes (*22*). Although preliminary, the extent of PS synthesis from PE under the same condition appeared to be minimal in comparison to PC by both liver and brain microsomes when tested for 18:0,22:6 species.

It has been demonstrated that membrane translocation and activation of PKCα (*35*, *36*) and Raf-1 kinase (*17*, *37*, *38*) are PS-concentration dependent. Concentration of PS may facilitate such membrane-related signaling processes particularly in neuronal membranes. It is conceivable that enrichment of 22:6n-3 in neuronal tissues may be a mechanism to maintain a high level of PS for an efficient signal transduction. During n-3 fatty acid deficiency where 22:6n-3 is depleted, an increase of 22:5n-6 in neuronal tissues may be warranted to minimize the loss of PS since the PC species containing this fatty acid is the best alternative as a substrate for PS synthesis.

In conclusion, using an in vitro assay method employing deuterium-labeled PC substrates and reversed-phase HPLC/electrospray mass spectrometry, we demonstrate that a tissue-specific substrate preference exists for the PS production. The preferential synthesis of 18:0,22:6-PS may be a mechanism supporting PS accumulation in neuronal tissues where 22:6n-3 is highly concentrated.

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BI035197X