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Phospholipid substrate-specificity of the L-serine base-exchange enzyme in rat liver microsomal fraction

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The specificity of the L-serine base-exchange enzyme towards the fatty acid composition of the phospholipid substrate was investigated with a rat liver microsomal fraction. The relative rates of L-serine incorporation into saturated–hexaenoic, saturated–pentaenoic, saturated–tetraenoic, saturated–trienoic, dienoic–dienoic, monoenoic–dienoic, saturated–dienoic and saturated–monoenoic + saturated–saturated phosphatidylserine molecular species were 42, 5, 23, 4, 5, 4, 5 and 11% respectively. This is similar to, but not identical with, the relative mass abundance of these molecular species in total liver cell phosphatidylserines. The results indicate that the substrate-specificity of the L-serine base-exchange enzyme can at least in part explain the observed fatty acid composition of rat liver phosphatidylserines.

The biosynthesis of phosphatidylserine in mammals takes place by an energy-independent base-exchange reaction between L-serine and preformed phospholipids (Borkenhagen *et al.*, 1961). This reaction requires Ca^{2+} ions (Hübscher, 1961), is localized in the microsomal fraction (Bjerve, 1973*a*), and both phosphatidylcholine and phosphatidylethanolamine as well as phosphatidylserine itself can serve as the phospholipid substrate (Dils & Hübscher, 1961). A minor part of phosphatidylcholine and phosphatidylethanolamine biosynthesis occurs through a similar base-exchange reaction with choline and ethanolamine (Bjerve, 1973*b*; Dils & Hübscher, 1961; Porcellati *et al.*, 1971). The base-exchange has been most extensively studied in rat liver and brain [see Kanfer (1980) and Baranska (1982) for recent reviews]. Kinetic studies have indicated the presence of two or more base-exchange enzymes in rat liver (Bjerve, 1973*a*), and three different enzymes with specificity towards choline, ethanolamine and L-serine respectively have been isolated from rat brain (Miura & Kanfer, 1976).

Approx. 45% of the phosphatidylserines in the rat liver cell are stearoyl-docosahexaenoyl species, and 30% are stearoyl-arachidonoyl species (Yeung *et al.*, 1977; Bjerve, 1982). It has been suggested that the arachidonoyl species are synthesized mainly by re-acylation of 1-acyl-*sn*-3-glycerophosphorylserine with arachidonoyl-CoA (Holub, 1980) by a de-acylation–re-acylation mechanism (Lands & Merkl, 1963). Except for that, little is known about the mechanisms controlling the fatty acid composition of phosphatidylserines in rat liver.

The present paper studies whether the L-serine base-exchange enzyme in rat liver microsomal fraction shows any substrate-specificity towards the phospholipid substrate, and to what extent such a specificity could explain the observed fatty acid composition of the phosphatidylserines.

Materials and methods

The microsomal fraction was prepared from female Wistar rats weighing 280–320g as described previously (Bjerve, 1971). Rats were killed by a blow on the neck, and the liver was rapidly excised and homogenized in 10vol. of 0.25M-sucrose. The microsomal fraction was finally suspended in 0.25M-sucrose, and used within 30min. All procedures were performed at 4°C. L-[U-^{14}C]-Serine (sp. radioactivity 176Ci/mol) was purchased from New England Nuclear, Boston, MA, U.S.A. Organic solvents (Merck, Darmstadt, Germany) were of analytical grade, and butylated hydroxytoluene (Koch–Light, Colnbrook, Bucks., U.K.) (50mg/l) was added as antioxidant when appropriate. Incubations (2.0ml total volume) were stopped by adding 7.5ml of chloro-

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form/methanol (1:2, v/v), and left at room temperature for 45min with occasional vortex-mixing before being filtered through sintered-glass discs. The residue was washed successively with 7.5ml of chloroform/methanol (1:2, v/v) and 5.0ml of chloroform, and the extracts were combined. Two phases were obtained by adding 7.0ml of 95mM-DL-serine in 50mM-HCl, and the lipid phase was finally washed once with 5.0ml of methanol/water (1:1, v/v). Radioactivity distribution between phospholipid classes was determined after separating them by t.l.c. (Skipski *et al.*, 1962).

To analyse the distribution of radioactivity within phosphatidylserine molecular species, a combined phosphatidylserine + phosphatidylinositol fraction was isolated by t.l.c. (Skipski *et al.*, 1962) and eluted (Arvidsson, 1968). The phosphatidylserine was modified by treatment with acetic acid anhydride and diazomethane to give the corresponding *N*-acetyl-*O*-methyl-phosphatidylserine (Bjerve, 1982). This derivative was further purified by t.l.c. on silica gel H (Merck) with chloroform/propan-2-ol (96:5, v/v) as solvent, which gave a complete separation from phosphatidylinositol. The *N*-acetyl-*O*-methyl-phosphatidylserines were separated according to their degree of unsaturation by 'argentation' chromatography (Bjerve, 1982). Fractions were localized in u.v. light after a spraying with 0.002% dichlorofluorescein in ethanol, and scraped into scintillation vials, to each of which was added 1.0ml of 1.7M-NaCl solution and 3 drops of conc. acetic acid (Webb & Mettrick, 1972) and then 10ml of Instagel (Packard Instruments, La Grange, IL, U.S.A.). For quantitative determination of the fractions separated by 'argentation' chromatography, they were eluted (Arvidsson, 1968) and, after the addition of heneicosaenoic acid (Sigma Chemical Co., St. Louis, MO, U.S.A.) as internal standard, transmethylated with 14% (v/v) BF_3 in methanol (Supelco, Bellefonte, PA, U.S.A.) (Metcalf & Schmitz, 1961). Quantitative determination of the methyl esters was performed by g.l.c. as described previously (Bjerve, 1982), and the results were corrected by using established response factors for the flame ionization detector.

Lipid phosphorus was determined by the method of Bartlett (1959) after digestion with 72% (w/v) $HClO_4$. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Liquid-scintillation counting was performed in a Packard Tri-Carb 3385 instrument.

Results and discussion

Table 1 shows the incorporation of L-[U-^{14}C]-serine into microsomal lipids. Some 60–80% of the radioactivity was incorporated into phosphatidylserine, and less than 1% was found in phosphatidylethanolamine. Most of the remainder was incorporated into phosphatidylcholine and sphingomyelin. In comparison, serine was incorporated exclusively into phosphatidylserine when the assay was performed at pH8.8 with a $CaCl_2$ concentration of 0.16mM (Bjerve, 1973*a*). The rate of incorporation into phosphatidylserine declined with time, whereas it remained nearly constant into phosphatidylcholine and sphingomyelin. The rate decreased similarly at both 0.5 μM and 55 μM L-serine concentrations. The latter serine concentration gave a 60-fold greater incorporation of L-serine into phosphatidylserine. This indicates that the decreased rate of synthesis is not due to a depletion of either L-serine or the phospholipid substrates, but probably reflects inactivation of the enzyme (Bjerve, 1973*a*). The microsomal fraction contained 6.4nmol of phosphatidylserine/mg of protein. In comparison, approx. 4nmol of L-serine

Table 1. *Incorporation of* L-[*U*-^{14}*C*]*serine into microsomal lipids*

Each incubation mixture contained 60mM-imidazole/HCl buffer, pH7.5, 4mM-$CaCl_2$ and 2.7mg of microsomal protein in a total volume of 2.0ml. The mixture in Expt. A contained 0.5μM-L-[U-^{14}C]serine (sp. radioactivity 396600 d.p.m./nmol), and that in Expt. B contained 55μM-L-[U-^{14}C]serine (sp. radioactivity 7180 d.p.m./nmol). Lipid extracts from three parallel incubations were pooled before analysis as described in the Materials and methods section. The results are those of one typical experiment. Abbreviations: PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; Sph, sphingomyelin + lysophosphatidylcholine.

		L-[U-^{14}C]Serine incorporated (nmol)				
	Incubation time (min)	PS	PE	PC	Sph	Neutral lipids
Expt. A	10	0.19	0.002	0.029	0.014	0.004
	20	0.24	0.002	0.073	0.042	0.004
	30	0.27	0.004	0.090	0.067	0.007
Expt. B	10	10.3	0.12	1.80	0.88	0.34
	20	15.0	0.19	2.38	2.45	0.41
	30	16.2	0.23	2.79	3.54	0.47

was incorporated into the microsomal phosphatidylserine fraction/min per mg of protein at a serine concentration of 55 μM. This indicates that the L-serine base-exchange enzyme has a rather high capacity for phosphatidylserine biosynthesis.

The incorporation of L-serine into different molecular species of phosphatidylserine is shown in Fig. 1. The saturated–hexaenoic phosphatidylserine species were most rapidly formed, and after 10 min contained 43% of the incorporated L-serine; the saturated–tetraenoic fraction contained 23%. The saturated–pentaenoic, saturated–trienoic, dienoic–dienoic, monoenoic–dienoic and saturated–dienoic fractions each contained 4–5%, and a combined saturated–monoenoic + disaturated fraction contained 11% of the incorporated L-serine. Incorporation reached a plateau with time similarly in all fractions, indicating that no specific molecular species of the phospholipid substrate had been exhausted. There was no difference in incorporation pattern between very low concentrations (Fig. 1*a*) and concentrations of L-serine near the K_m of the base-exchange enzyme (Fig. 1*b*).

Table 2 shows the relative mass abundance of the microsomal phosphatidylserine subspecies. As in total liver phosphatidylserine, the saturated–hexaenoic fraction dominated, containing 31.3% of the total. The contents of the disaturated and saturated–monoenoic fractions were 17% and 14% respectively. In comparison, the sum of these two fractions was 3.9% in total liver phosphatidylserines (Bjerve, 1982).

The present results show that the base-exchange reaction primarily forms hexaenoic and tetraenoic phosphatidylserine species, but also forms a considerable amount of disaturated and saturated–monoenoic species. This could reflect either the availability of the corresponding phospholipid substrates or a specificity of the L-serine base-exchange enzyme towards the fatty acid composition of the phospholipid substrate. Both phosphatidylethanolamine and phosphatidylcholine as well as phosphatidylserine itself are substrates in this reaction, but their relative importance is not known. Nevertheless, it seems that considerably more hexaenoic species are formed than can be accounted for by the composition of the available phospholipid substrates alone (Bjerve, 1971, 1982), which indicates that there is a specificity of the L-serine base-exchange enzyme towards the fatty acid composition of the phospholipid substrate. Taki & Kanfer (1978) also suggested that a purified L-serine base-exchange enzyme from rat brain showed some specificity towards the fatty acid composition of the lipid substrate.

The observed rate of synthesis of the different molecular species of phosphatidylserine is somewhat different from the composition of molecular species found both in microsomal (Table 2) and in total liver phosphatidylserines (Bjerve, 1982). This

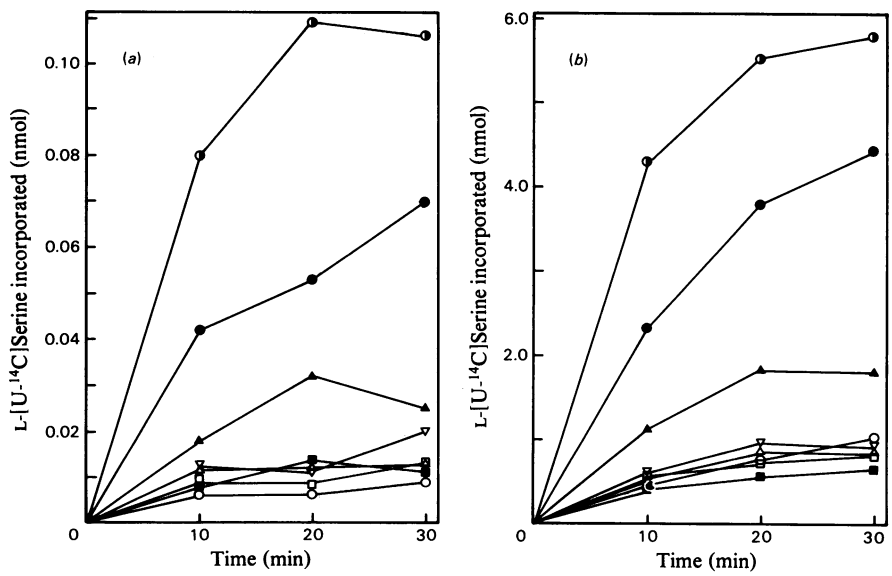

Fig. 1. *Incorporation of* L-[*U*-^{14}C]*serine into phosphatidylserine molecular species*
Incubations were performed as described in Table 1 legend. The results are those of one typical experiment. (*a*) 0.5 μM-L-[U-^{14}C]serine; (*b*) 55 μM-L-[U-^{14}C]serine. Phosphatidylserine molecular species: ◑, saturated–hexaenoic; □, saturated–pentaenoic; ●, saturated-tetraenoic; ■, saturated-trienoic; △, dienoic–dienoic; ○, monoenoic–dienoic; ▽, saturated–dienoic; ▲, saturated–monoenoic + saturated–saturated.

Table 2. *Composition of rat liver microsomal phosphatidylserine subclasses*

Phosphatidylserine was isolated and subfractionated as described in the Materials and methods section. The microsomal preparation contained 6.4 nmol of phosphatidylserine/mg of protein (range 3.5–9.3 nmol/mg). Results are given as percentages of total phosphatidylserine, and they are given as means ± S.E.M. (ranges in parentheses) for four separate experiments. Abbreviations for phosphatidylserine molecular species: SS, saturated–saturated; SM, saturated–monoenoic; SD, saturated–dienoic; MD, monoenoic–dienoic; DD, dienoic–dienoic; STri, saturated–trienoic; ST, saturated–tetraenoic; SP, saturated–pentaenoic; SH, saturated–hexaenoic.

Fraction	Composition (%)
SS	17.1 ± 2.5 (12.2–20.2)
SM	14.0 ± 1.4 (11.2–15.7)
SD	5.5 ± 0.5 (4.4–6.7)
MD + DD	7.0 ± 0.6 (6.1–8.6)
STri + ST	16.0 ± 0.8 (13.9–17.4)
SP	8.2 ± 1.5 (5.2–12.0)
SH	31.3 ± 2.6 (27.1–38.2)

suggests that phosphatidylserine is either deacylated and subsequently re-acylated as described by Lands & Merkl (1963), or that some species of phosphatidylserine are predominantly formed by a different pathway. Acylation of 1-acyl-*sn*-glycero-3-phosphorylserine with rat liver microsomal fraction showed that palmitoyl-CoA and stearoyl-CoA were only 10% as efficient acyl-donors as linoleyl-CoA and arachidonoyl-CoA (Holub, 1980). As 30% and 50% of the microsomal and total liver phosphatidylserines respectively are saturated–hexaenoic species, and as they also contain 15 times more stearic acid than palmitic acid (Yeung *et al.*, 1977; Bjerve, 1982), this indicates that the Lands & Merkl (1963) pathway does not play any quantitatively significant role in the biosynthesis of rat liver phosphatidylserines.

Other, energy-requiring, pathways of phosphatidylserine synthesis have been suggested to explain some observed phenomena in systems investigated *in vitro* (Hübscher *et al.*, 1959; Bygrave & Bücher, 1968; Baranska, 1980). It has also been reported that pyrophosphatidic acid might be an immediate precursor of phosphatidylserine biosynthesis in an Ni^{2+}-and-ATP requiring pathway described in rat brain microsomal fraction (Pullarkat *et al.*, 1981). It remains to be established whether any of these other possible pathways might contribute to the observed fatty acid composition of rat liver phosphatidylserines.

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