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A new pathway for phosphatidylserine synthesis in rat liver microsomes

Jolanta Barańska

Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warszawa, Poland

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It has been shown that the phosphate moiety of glycerophosphate is incorporated into phosphatidylserine of rat liver microsomes, but not of mitochondria. The reaction is dependent on CMP. This observation suggests that the new pathway of acyl-specific synthesis of phosphatidylserine proposed by J.P. Infante [(1984) FEBS Lett. 170, 1–14] can proceed in rat liver microsomes.

Phosphatidylserine biosynthesis; Phosphatidylcholine; Microsome; Mitochondria; (Rat liver)

1. INTRODUCTION

A new hypothetical pathway for the synthesis of various phospholipids containing specific fatty acid residues has been proposed recently by Infante [1]. According to this scheme (exemplified for phosphatidylserine in fig.1), the 'storage' form of the phospholipid reacts with CMP to give rise to diacylglycerol and the corresponding CDP-base (reaction 2). Subsequently, the base moiety from the latter compound is transferred to glycerol 3-phosphate to produce glycerophosphorylserine (reaction 3) which is then acylated with specific fatty acids (reactions 4 and 5) to an acyl-specific form of the same phospholipid class. In this metabolic sequence glycerophosphorylserine is an intermediate, and the phosphate moiety of phosphatidylserine originates from glycerophosphate. More recently, the occurrence of this pathway for phosphatidylcholine and phosphatidylethanolamine has been experimentally documented in post-nuclear supernatant of various animal tissues by the same author [2–4].

Correspondence address: J. Barańska, Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warszawa, Poland

The present study was undertaken to examine this pathway for phosphatidylserine by using labelled glycerophosphate in the protocol design. This paper provides evidence in support of Infante's hypothesis for acyl-specific synthesis of phosphatidylserine in rat liver microsomes. In mitochondria, however, the acyl-specific phosphatidylserine formation was not observed. These results are discussed in relation to previous data [5], which show the ATP- and CMP-stimulated incorporation of labelled serine into mitochondrial phospholipids.

2. MATERIALS AND METHODS

Liver microsomes and mitochondria from Wistar rats were isolated by a conventional procedure [6].

Microsomes loaded with phosphatidylserine were prepared according to Bjerve [7], by incubating the particles (about 10 mg protein) in the medium containing 60 mM imidazol buffer (pH 7.4), 4 mM $CaCl_2$ and 2 mM L-serine in a final volume of 1.0 ml (medium A). After 30 min at 37°C, 10 mM EGTA was added and the particles were incubated further in the medium containing 60 mM KCl, 10 mM Tris-HCl (pH 7.4), 7 mM ATP, 6 mM $MgCl_2$, 2 mM NaN_3, 0.3 mM sodium palmitate, 0.2 mM CoA-SH (medium B), with 0.5 mM *sn*-glycerol 3-[^{32}P]phosphate (about 1×10^5 cpm), and with or without 2 mM CMP. Incubation was carried out for 30 min at 37°C in a final volume of 1.0 ml. The amount of added microsomes, loaded with phosphatidylserine, or freshly prepared, corresponded to 10 mg protein. Microsomes which

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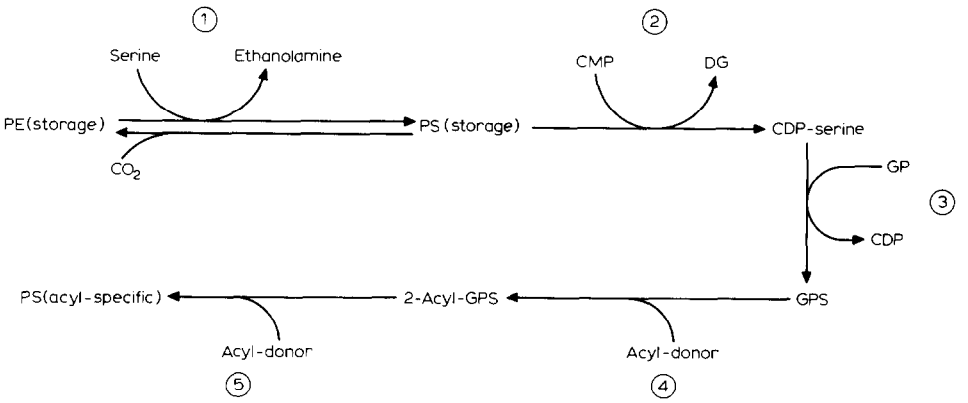

Fig.1. Pathway for the biosynthesis of acyl-specific phosphatidylserine according to Infante [1]. PE, phosphatidylethanolamine; PS, phosphatidylserine; DG, *sn*-1,2-diacylglycerol; CDP-serine, cytidinephosphoserine; GP, glycerol 3-phosphate; GPS, glycerophosphorylserine.

were not loaded with phosphatidylserine were treated with 10 mM EGTA before incubation in medium B.

Rat liver mitochondria (about 6 mg protein) were incubated for 1 h at 37°C in 1.0 ml of medium B, with 2 mM CMP, with or without 1 mM L-serine, and with 1 mM *sn*-glycerol 3-[^{32}P]phosphate (0.6×10^6 cpm). In the control sample, mitochondria were incubated with cold 1 mM *sn*-glycerol 3-phosphate and 0.1 mM L-[3-^{14}C]serine (spec. act. 57 Ci/mol).

All incubations were terminated by addition of methanol/chloroform (2:1, v/v), and lipids were extracted according to Bligh and Dyer [8], with the modification of Bjerve [9]. After extraction of lipids, phospholipids were separated and identified by thin-layer chromatography on silica gel H and G, in chloroform/methanol/acetic acid/water (65:25:8:4, v/v), and chloroform/methanol/water (65:25:4, v/v) system [10], respectively. Spots were visualized using iodine vapour, ninhydrin and autoradiography.

rac-Glycerol 3-[^{32}P]phosphate was prepared by heating inorganic [^{32}P]phosphate with glycerol [11]. [U-^{14}C]-Phosphatidylserine, used as a standard for thin-layer chromatography and autoradiography was synthesized by incubating rat liver microsomes with [U-^{14}C]serine and subsequently separated on a CM-cellulose column [12]. L-[3-^{14}C]Serine and [U-^{14}C]serine were from Amersham (Amersham, England), whereas [^{32}P]phosphate was from the Institute of Nuclear Research (Świerk, Poland).

Radioactivity was measured with a scintillation spectrometer making use of the Čerenkov effect for ^{32}P [13] or using liquid scintillation cocktails for ^{14}C. Proteins were determined by the biuret method [14].

3. RESULTS

Table 1 shows the formation of phosphatidylserine and phosphatidylcholine in rat liver microsomes as measured by *sn*-glycerol 3-[^{32}P]-phosphate incorporation. As observed, the reaction is increased upon addition of CMP; in its absence the level of both synthesized phospholipids is about half of that in the complete medium. Table 1 also shows that phosphatidylserine synthesis is distinctly increased when microsomes previously loaded with cold phosphatidylserine are used.

When mitochondria were incubated with *sn*-glycerol 3-[^{32}P]phosphate and CMP under similar conditions no radioactivity in the phosphatidylserine spot was found. The main labelled phospholipids were phosphatidic acid, phosphatidylglycerol and lysophosphatidic acid (fig.2). Addition of cold L-serine to the incubation medium did not result in the appearance of a [^{32}P]phosphatidylserine spot either (fig.2, line 3). The same results were obtained when separation of phos-

Table 1

Incorporation of *sn*-glycerol 3-[^{32}P]phosphate into rat liver microsomal phosphatidylserine and phosphatidylcholine

Phospholipid	Microsomes non-loaded with phosphatidyl-serine + CMP	Microsomes loaded with phosphatidylserine	
		+ CMP	− CMP
Phosphatidyl-serine	0.24	0.40	0.18
Phosphatidyl-choline	0.60	0.50	0.26

Data in nmol phospholipid/mg protein per 60 min incubation in conditions described in section 2

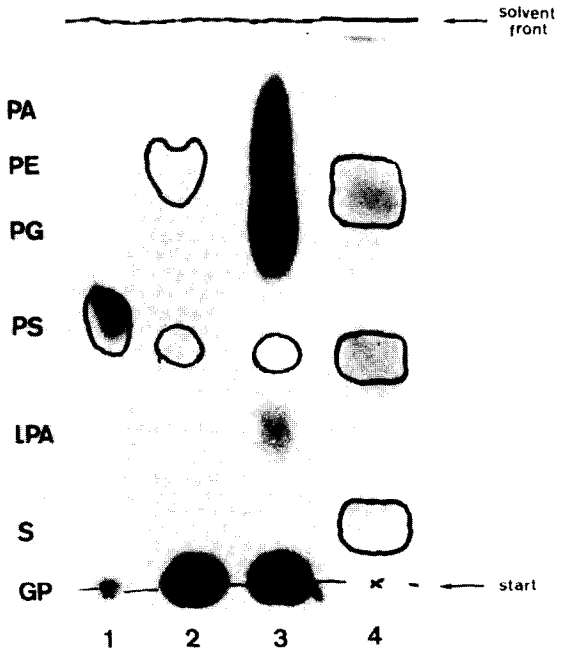

Fig.2. Incorporation of *sn*-glycerol 3-[^{32}P]phosphate and L-[3-^{14}C]serine into mitochondrial phospholipids. Rat liver mitochondria were incubated in medium B as described in section 2. After incubation, lipids were extracted and phospholipids separated on Silica gel H in chloroform/methanol/acetic acid/water (65:25:8:4, v/v). The spots of phosphatidylserine and phosphatidylethanolamine were visualized by the positive reaction with 0.1% ninhydrin (drawn outlines; the spot of phosphatidylethanolamine in line 3 is not marked on the autoradiogram). The radioactive spots were visualized by autoradiography. Line 1, standard of [U-^{14}C]phosphatidylserine (70000 cpm applied to the plate); line 2, zero time control (30000 cpm applied to the plate); line 3, 60 min of incubation with labelled glycerol 3-phosphate and cold serine (70000 cpm applied to the plate); line 4, 60 min of incubation with L-[3-^{14}C]serine (60000 cpm applied to the plate). PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PS, phosphatidylserine; LPA, lysophosphatidic acid; S, serine; GP, glycerophosphate.

pholipids was performed by thin-layer chromatography in chloroform/methanol/water system [10]. Contrary to the result obtained with labelled glycerophosphate, and in agreement with our previous data [5], L-[3-^{14}C]serine was incorporated into phosphatidylserine and decarboxylated to phosphatidylethanolamine (fig.2, line 4). Incorporation of [3-^{14}C]serine into mitochondrial phospholipids amounted to 0.16 nmol/mg protein per h, and the radioactivity incorporated into phosphatidylserine and phosphatidylethanolamine amounted to 33% and 38% of the total label, respectively.

4. DISCUSSION

Phosphatidylserine in microsomes of animal tissues is formed by the base-exchange reaction and is believed not to be synthesized from free serine and CDP-diacylglycerol, the pathway described for bacteria (cf. [15]). It is also generally believed that phosphatidylserine is not synthesized in a similar way to the incorporation of ethanolamine and choline in animal tissues, i.e. via the cytidine pathway with CDP-serine as intermediate. In the pathway proposed by Infante [1], CDP-serine is assumed to be synthesized by the transfer of phosphorylserine from non-acyl-specific phosphatidylserine to CMP, thus in a different way to that of CDP-derivatives in the cytidine pathway.

In the present study, the ability of microsomes to synthesize CDP-serine and glycerophosphorylserine was not examined. However, the entry of the label from *sn*-glycerol 3-[^{32}P]phosphate into phosphatidylserine and the stimulation of this process by CMP provide evidence that this phospholipid can be formed according to the scheme proposed by Infante [1]. Nevertheless, this metabolic sequence operating with endogenous phosphatidylserine as the primary substrate leads only to the modification of the pre-existing phospholipid and not to its de novo synthesis. This is shown in table 1, which demonstrates that enrichment in endogenous phosphatidylserine caused a distinct increase of the level of the radioactive final product. A negligible amount of phosphatidylserine present in mitochondrial membranes could be one of the reasons why the acyl-specific phospholipid formation in these particles was not observed.

Infante [1] suggested that the acyl-specific phosphatidylserine pathway explains the literature data for the incorporation of serine into mitochondrial phospholipids stimulated by ATP, CMP, CoA-SH, Mg^{2+}, and glycerophosphate. This process, first reported by Hübscher et al. [16,17], was corroborated later by other authors [18,19] and myself [5]. I searched for a mechanism that could explain this reaction and concluded that none of the known pathways for phosphatidylserine

biosynthesis could apply. The pathway of Infante [1] explains the requirement for these reactants but does not explain the incorporation of labelled serine into phospholipids. It was also shown that the radioactivity from neither [^{32}P]phosphatidic acid nor [β,γ-^{32}P]ATP was recovered in phosphatidylserine [5]. The present study demonstrates that ^{32}P from glycerophosphate was not incorporated into mitochondrial phosphatidylserine either. Thus, the mechanism of ATP- and CMP-stimulated serine incorporation remains still unclear.

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