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Biochimica et Biophysica Acta 1761 (2006) 313–323
BIOCHIMICA ET BIOPHYSICA ACTA
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Phospholipid homeostasis in phosphatidylserine synthase-2-deficient mice

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Received 10 January 2006; received in revised form 4 March 2006; accepted 8 March 2006
Available online 31 March 2006

Abstract

Phosphatidylserine (PS) is synthesized in mammalian cells by two distinct serine-exchange enzymes, phosphatidylserine synthase-1 and -2. We recently demonstrated that mice lacking PS synthase-2 develop normally and exhibit no overt abnormalities [Bergo et al., (2002) J. Biol. Chem. 277:47701–47708]. We now show that PS synthase-2 mRNA levels are up to 80-fold higher in livers of embryos than in adults. Despite reduced serine-exchange activity in several tissues of PS synthase-2 deficient mice, the phospholipid composition of mitochondria and microsomes from these tissues is normal. Although PS synthase-2 is highly expressed in neurons, axon extension of cultured sympathetic neurons is not impaired by PS synthase-2 deficiency. We hypothesized that mice compensate for PS synthase-2 deficiency by modifying their phospholipid metabolism. Our data show that the rate of PS synthesis in hepatocytes is not reduced by PS synthase-2 deficiency but PS synthase-1 activity is increased. Moreover, PS degradation is decreased by PS synthase-2 deficiency, probably as a result of decreased PS degradation via phospholipases rather than decreased PS decarboxylation. These experiments underscore the idea that cellular phospholipid composition is tightly controlled and show that PS synthase-2-deficient hepatocytes modify phospholipid metabolism by several compensatory mechanisms to maintain phospholipid homeostasis.

Keywords: Phosphatidylserine; Phosphatidylethanolamine; Phosphatidylserine synthase; Phosphatidylserine decarboxylase; Hepatocytes; Knock-out mice

1. Introduction

Phosphatidylserine (PS) is an aminoglycerophospholipid that constitutes 5–10% of mammalian membrane phospholipids. PS is synthesized in mammals by two distinct serine-exchange enzymes, phosphatidylserine synthase-1 (PSS1) and phosphatidylserine synthase-2 (PSS2) (reviewed in [1]). These enzymes are structurally related, with 32% amino acid identity, and each is predicted to contain several transmembrane domains [2–5]. PSS1 and PSS2 catalyze the exchange of serine with the head-group of a pre-existing phospholipid [6], but the two enzymes differ in their substrate specificity. Whereas PSS1 uses phosphatidylcholine (PC) for the exchange reaction [7,8], PSS2 uses phosphatidylethanolamine (PE) (Fig. 1) [9]. In Chinese hamster ovary (CHO) cells and rat liver, serine-exchange activity is associated with the endoplasmic reticulum (ER) and is enriched 2- to 4-fold in an ER sub-fraction, the mitochondria-associated membranes (MAM), compared to the bulk of the ER [10]; indeed, PSS1 and PSS2 are localized to MAM and largely excluded from the bulk of ER [11]. MAM have been proposed to be a specialized domain of the ER that associates with mitochondria and mediates the import of newly-synthesized PS into mitochondria in mammalian cells [12] and yeast [13,14]. A major metabolic fate of PS is its conversion to PE in mitochondria by PS decarboxylase [15,16]. Essentially all PE in mitochondria is derived from the decarboxylation of PS that is imported into mitochondria from MAM, rather than from PE produced from the CDP-ethanolamine pathway in the ER [12,17,18]. The importance of the PS decarboxylation pathway is underscored by our recent finding that disruption of the PS

Abbreviations: CHO, Chinese hamster ovary; ER, endoplasmic reticulum; MAM, mitochondria-associated membranes; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PSS, phosphatidylserine synthase

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doi:10.1016/j.bbalip.2006.03.005

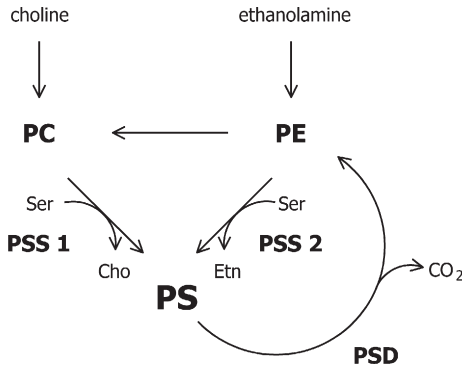

Fig. 1. Schematic view of PS biosynthetic pathways in mammals. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PSS1, PS synthase-1; PSS2, PS synthase-2; PSD, PS decarboxylase; Ser, serine; Cho, choline; Etn, ethanolamine.

decarboxylase gene in mice causes embryonic lethality and severe mitochondrial defects [19].

The reason why mammals have two different enzymes for PS synthesis is not clear. We hypothesized that PSS1 and PSS2 might generate distinct pools of PS that are used for different purposes. In mice, PSS2 mRNA is particularly highly expressed in Sertoli cells of the testis and Purkinje neurons in the brain, whereas PSS1 mRNA is more ubiquitously expressed [20,21]. CHO cell mutants have been generated that are deficient in either PSS1 or PSS2, or both PS synthases. In PSS1-deficient CHO cells, PS synthesis is reduced by 35–55% and the mass of PS and PE is correspondingly reduced [7,8]; these cells require supplementation with PS, PE or ethanolamine for normal growth. The PSS1-deficient cells were further mutagenized to generate cells that were deficient in both PSS1 and PSS2. The double mutant cells are PS auxotrophs [9] and PS synthase activity is 95% lower than in wild-type cells; the remaining 5% of PS synthase activity is likely contributed by residual PSS2 activity. The double mutant cells were transfected with a cDNA encoding PSS1 to generate cells that lacked PSS2 but expressed PSS1 [9]. These cells were viable when supplemented with either ethanolamine or PS. Neither PSS1 nor PSS2 is specifically required by CHO cells for exposure of PS on the cell surface during apoptosis [22].

To determine whether or not PSS2 is required for normal development and function in mice, PSS2-deficient mice were generated in our laboratories [21]. These mice develop normally and are viable, although males have smaller testes and ~10% of male mice exhibit testicular atrophy. In addition, serum levels of follicle-stimulating hormone in male *Pss2*$^{-/-}$ mice are higher than in *Pss2*$^{+/+}$ mice, suggesting Sertoli cell dysfunction. Remarkably, the phospholipid composition of all mouse tissues examined was unaltered by PSS2 deficiency, despite the finding that in some tissues, particularly the testis, the specific activity for serine-exchange was markedly lower than in wild-type mice. The in vitro enzymatic assay for serine-exchange measures the incorporation of radiolabeled serine into PS and, therefore, reflects the combined activities of PSS1 and PSS2. Consequently, in PSS2-deficient mice the residual serine-exchange activity from PSS1, probably in combination with compensatory changes in phospholipid metabolism, is apparently sufficient to maintain normal tissue levels of PS and PE.

These observations led us to determine if adaptive changes in phospholipid metabolism occur in *Pss2*$^{-/-}$ mice. Our experiments show that in primary hepatocytes from *Pss2*$^{-/-}$ mice, the rate of PS synthesis is the same as in *Pss2*$^{+/+}$ hepatocytes, whereas the rate of PS degradation is decreased. Moreover, the flux through several phospholipid biosynthetic pathways is modestly altered by PSS2 deficiency in a gender-specific manner. Thus, several compensatory modifications in phospholipid metabolism are implemented in *Pss2*$^{-/-}$ mice to maintain phospholipid homeostasis.

2. Materials and methods

2.1. Maintenance of *Pss2*$^{+/+}$ and *Pss2*$^{-/-}$ mice and isolation of primary hepatocytes

Pss2$^{+/+}$ and *Pss2*$^{-/-}$ mice were generated as described previously [21]. The mice were of mixed genetic background (50% C57Bl6 and 50% 129/OlaHsd) and the colony was maintained at the University of Alberta. Mice were weaned at 21 days of age, housed in a barrier facility with a 14-h light, 10-h dark cycle, and fed a chow diet containing 4.5% fat (LabDiet, Richmond, IN). For studies on embryonic development, pregnant females were euthanized and embryos were removed from the uterus. For experiments in which mice were fed a choline-deficient diet, the mice were placed in wire-bottomed cages with no bedding and were fed a semi-purified choline-deficient, or choline-supplemented, diet for 3 weeks [23].

For isolation of primary hepatocytes, mice were anaesthetized and a mid-line incision was made. Hank's EGTA solution containing 10 μg/ml insulin was perfused through the portal vein until the liver was clear of blood. The superior and inferior vena cava were tied and the perfusion was continued with Hank's collagenase solution (100 units/ml) containing 10 μg/ml insulin until the liver became soft (~3 min). The liver was removed, cut into pieces, transferred to Hank's collagenase solution and mixed until all clumps of tissue dissipated. The hepatocytes were suspended in medium containing 10% fetal bovine serum and plated on collagen-coated 60 mm dishes (2×10^6 cells/dish). Cell viability (typically >90%) was estimated by Trypan blue exclusion. After the hepatocytes had attached to the dish (2 to 3 h) the medium was removed and cells were washed, then cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, and used within 24 h of isolation.

2.2. Analysis of mRNA levels by real-time qPCR

Mouse tissue (50 to 100 mg) was homogenized with three 10 s bursts with a polytron on ice in TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA was extracted according to manufacturer's instructions. RNA quality was confirmed by electrophoresis on 1.5% formaldehyde agarose gels and by measurement of the ratio of 28S/18S ribsomal RNA. Total RNA (5 μg) was reverse-transcribed in a 20-μl volume containing oligo(dT) and Superscript II enzyme (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Primers used for the reference gene, cyclophilin A, were 5′-TCC AAA GAC AGC AGA AAA CTT TCG-3′ and 5′-TCT TCT TGC TGG TCT TGC CAT TCC-3′. For PS decarboxylase, the primers were 5′-CAA CCT CAG CGA GTT CTT CC-3′ and 5′-CCT GCT CCA CCT CAG AGT TC-3′. For PS synthase-2, the primers were 5′-ACT GTG CTG TTC ATC CTC ACC-3′ and 5′-AAA TGG CCC GTC TTT AGC-3′. For PS synthase-1, the primers were 5′-CCT TGT TGA TCC GTA GTT ATG GG-3′ and 5′-TGC CCA GTG GTA AGT TCT CAT CTC-3′. The 20 μl PCR reaction contained 100 ng cDNA, 10 μl Platimum SybrGreen qPCR Supermix UDG (Invitrogen, Carlsbad, CA), and 1.6 μM cyclophilin primers or 3.0 μM PS decarboxylase or PSS2 primers. Real-time qPCR was performed with a Rotor-Gene RG-3000 thermocycler (Corbett Research, Mortlake, NSW, Australia) with samples from at least three mice and each sample was analyzed in triplicate. Data were normalized to the reference gene with the Pfaffl method [24].