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Review

Formation and function of phosphatidylserine and phosphatidylethanolamine in mammalian cells ☆

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ABSTRACT

Phosphatidylserine (PS) and phosphatidylethanolamine (PE) are metabolically related membrane aminophospholipids. In mammalian cells, PS is required for targeting and function of several intracellular signaling proteins. Moreover, PS is asymmetrically distributed in the plasma membrane. Although PS is highly enriched in the cytoplasmic leaflet of plasma membranes, PS exposure on the cell surface initiates blood clotting and removal of apoptotic cells. PS is synthesized in mammalian cells by two distinct PS synthases that exchange serine for choline or ethanolamine in phosphatidylcholine (PC) or PE, respectively. Targeted disruption of each PS synthase individually in mice demonstrated that neither enzyme is required for viability whereas elimination of both synthases was embryonic lethal. Thus, mammalian cells require a threshold amount of PS. PE is synthesized in mammalian cells by four different pathways, the quantitatively most important of which are the CDP-ethanolamine pathway that produces PE in the ER, and PS decarboxylation that occurs in mitochondria. PS is made in ER membranes and is imported into mitochondria for decarboxylation to PE via a domain of the ER [mitochondria-associated membranes (MAM)] that transiently associates with mitochondria. Elimination of PS decarboxylase in mice caused mitochondrial defects and embryonic lethality. Global elimination of the CDP-ethanolamine pathway was also incompatible with mouse survival. Thus, PE made by each of these pathways has independent and necessary functions. In mammals PE is a substrate for methylation to PC in the liver, a substrate for anandamide synthesis, and supplies ethanolamine for glycosylphosphatidylinositol anchors of cell-surface signaling proteins. Thus, PS and PE participate in many previously unanticipated facets of mammalian cell biology. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.

1. Introduction

Phosphatidylserine (PS) and phosphatidylethanolamine (PE) are two metabolically related aminophospholipids (Fig. 1) that are present in membranes of all eukaryotic and prokaryotic cells (reviewed in Ref. [1]). In mammalian, plant and yeast cells phosphatidylcholine (PC) is the most abundant phospholipid whereas PE is the second most abundant. However, with a few exceptions, prokaryotes do not make PC so that in this class of organisms PE is usually the most abundant phospholipid. In eukaryotic cells, PE and PS account for approximately 20% and 3–15%, respectively, of total phospholipids. The majority of phospholipids in mammalian cells are made in the ER whereas mitochondria supply all of the cardiolipin and a significant fraction of PE. Since mitochondria are presumed to be derived from bacteria during the evolution of eukaryotic cells, it has been suggested that the lack of PC synthesis in mitochondria reflects the (general) inability of bacteria to synthesize PC. On the other hand, PE is the major bacterial phospholipid so that mammalian mitochondria have retained the capacity for synthesis of PE, as well as another abundant bacterial phospholipid cardiolipin. Intriguingly, however, mammalian mitochondria do not make PS whereas bacteria do, albeit by a pathway different from that in mammalian cells. Thus, not all of the phospholipid biosynthetic capacity of bacteria has been retained in mammalian mitochondria.

PE was first isolated from the brain as “cephalin” by Ludwig Thudichum in 1884. His research on this topic was for many years considered to be “relatively insignificant,” according to Thudichum's obituaries in Nature [volume 64, page 527 (1901)] and “The Times” of London (Sept. 10, 1901). The latter article stated that “the knowledge yielded by these researches was hardly commensurate with the time and cost at which it was obtained.” Unfortunately, similar sentiments are even today often applied to basic research. Almost 70 years later (1952) the structure of PE was deduced by Baer and colleagues [2]. In 1941 PS was identified as a secondary component

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

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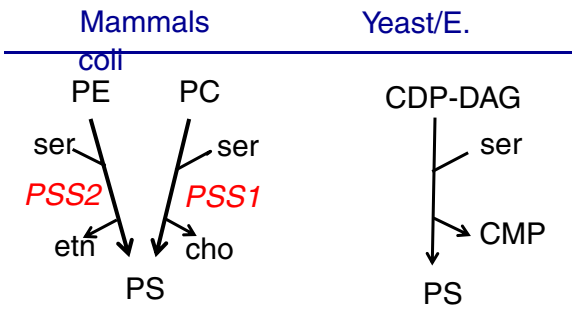

Fig. 1. Phosphatidylserine (PS) biosynthesis in mammals and yeast. In mammalian cells PS is synthesized by calcium-dependent base-exchange reactions between a pre-formed phospholipid and L-serine (ser). PS synthase-1 (PSS1) catalyzes the exchange of choline (cho) in phosphatidylcholine (PC) for serine, whereas PSS2 catalyzes the exchange of serine for ethanolamine (etn) in phosphatidylethanolamine (PE). The PS biosynthetic pathway in yeast and prokaryotes is completely different from that in mammalian cells. CDP-diacylglycerol and serine react to produce PS in a reaction catalyzed by a PS synthase unrelated to that in mammalian cells.

of cephalin which was originally thought to be pure PE. The structure of PS was elucidated by Folch in 1941 [3] and was subsequently confirmed by chemical synthesis [4].

In this article we shall discuss the mechanisms of the biosynthesis and cellular functions of PS and PE, particularly in mammalian cells.

2. Phosphatidylserine

2.1. Functions of phosphatidylserine

PS is not equally abundant in membranes of all types of mammalian cells or tissues. Compared to other tissues, the brain, and particularly the retina, is enriched in PS and PE. Moreover, in the human brain >36% of the acyl-chains of PS consist of docosahexanoyl residues [5,6] and the presence of these acyl-chains appears to be essential for normal functioning of the nervous system [6–9]. The concentration of PS also varies among different organelle membranes (reviewed in Ref. [10]); in general, the PS concentration is highest in plasma membranes and endosomes, but is very low in mitochondria, particularly in mitochondrial inner membranes. In addition, PS is not symmetrically distributed across the two leaflets of the membrane bilayer: PS is normally highly enriched in the inner, compared to the outer, leaflet of the plasma membrane, whereas the choline-containing lipids, PC and sphingomyelin, are enriched in the outer leaflet [11–13]. Studies on the erythrocyte membrane indicate that >96% of PS resides on the inner leaflet of the bilayer [14]. It should be noted, however, that little conclusive information is available on the transbilayer distribution of phospholipids such as PS in organelles other than the plasma membrane.

2.2. Transbilayer movement of PS in the plasma membrane

The initiation of several important physiological processes causes a redistribution of PS from the inner, to the outer, surface of the plasma membrane of mammalian cells. For example, during the blood-clotting cascade, the transbilayer asymmetry of PS in the plasma membrane of activated platelets is markedly altered so that PS becomes exposed on the cell surface. Consequently, the clotting factors V, VIII, X and prothrombin are recruited to the surface of platelets so that blood coagulation is promoted [14–17]. Similarly, during sperm maturation, the asymmetric distribution of PS in the plasma membrane is dissipated and PS becomes exposed on the surface of the sperm [18].

A particularly well-characterized process is the transbilayer movement of PS from the inner to the outer leaflet of the plasma membrane during the early stages of apoptosis. The exposure of PS on the surface of apoptotic cells represents a signal for the recognition and engulfment of these cells via PS receptors that are expressed on the surface of phagocytic cells [19–23]. Several candidate PS receptors have been identified on macrophages [22–27]. Consequently, the release of potentially toxic molecules from the dying cells is prevented. Some of the PS exposed during apoptosis was reported to be newly synthesized [28]. Nevertheless, in cells in which total PS biosynthetic activity was reduced by 95% [29], apoptosis progressed normally and PS exposure on the cell exterior during apoptosis was not compromised [30]. In addition, the exposure of PS on the surface of red blood cells serves as a signal for eryptosis (the clearance of red blood cells from the circulation by phagocytic cells) [31,32]. Moreover, not only PS, but the PS hydrolysis product, lyso-PS, is exposed on the surface of activated and dying neutrophils thereby initiating the clearance of these cells during acute inflammation [33,34]. The cell surface exposure of lyso-PS has also been reported to be a signal for the activation of mast cells and for platelet degranulation [35,36].

The mechanisms by which the asymmetric transbilayer distribution of PS in the plasma membrane is established, maintained and dissipated have been extensively investigated. ATP-dependent aminophospholipid (PS) flippase activities have recently been identified in yeast as members of the P-type ATPase family of transporters (i.e. Atp8a1 and Drs2 [37,38]). Orthologs of these proteins are likely to play similar roles in mammals since elimination of Atp8a1 in mice dramatically increased the externalization of PS in hippocampal neurons, and even impaired hippocampal learning [39]. Another protein that has been proposed to mediate the transbilayer movement of PS in the plasma membrane is the calcium-dependent protein, scramblase-1, that can randomize the distribution of phospholipids such as PS across membrane bilayers [40]. During apoptosis, the intracellular concentration of calcium increases so that the aminophospholipid translocase activity is inhibited whereas the scramblase is activated. Consequently, it has been proposed that these complementary changes induce the translocation of PS to the external leaflet of the plasma membrane during apoptosis [21]. However, whether or not scramblase-1 does indeed function as an aminophospholipid translocase in the plasma membrane remains unclear since targeted deletion of scramblase-1 in mice did not reduce the transbilayer movement of phospholipids in the plasma membrane [41].

2.3. Intracellular functions of PS

Although the extracellular functions of PS have been most extensively studied, PS also participates in many intracellular processes. A key function of PS is as the precursor of PE via the mitochondrial enzyme PS decarboxylase; this function of PS will be discussed in detail in Section 5.4).

Many of the intracellular functions of PS appear to depend on its anionic nature. For example, some key signaling proteins, such as the tyrosine kinase Src, as well as the Ras and Rho family of GTPases, contain positively-charged motifs that bind to PS, thereby contributing to the membrane targeting and activation of these proteins [42–45]. A recently discovered function of PS is its ability to target proteins to phagosomes ([46]; reviewed in Ref. [47]). In these studies, the PS-binding C2 domains of GFP-tagged discoidin and lactadherin were expressed intracellularly and used to assess the intracellular distribution of PS, particularly during phagocytosis (reviewed in Ref. [47]). These studies demonstrated that the highest concentration of PS is in the plasma membrane and endocytic organelles, and also indicated that PS is enriched in the luminal, compared to the cytosolic, leaflet of the ER [48].

PS also modifies the catalytic activity of several key signaling proteins that contain C2 domains, such as synaptotagmin, dynamin-1 [49], protein kinase C [50] and Annexin V [51]. In addition, the specific binding of PS to the PH domain of evectin-2 in recycling endosomes appears to be required for retrograde membrane trafficking [52].