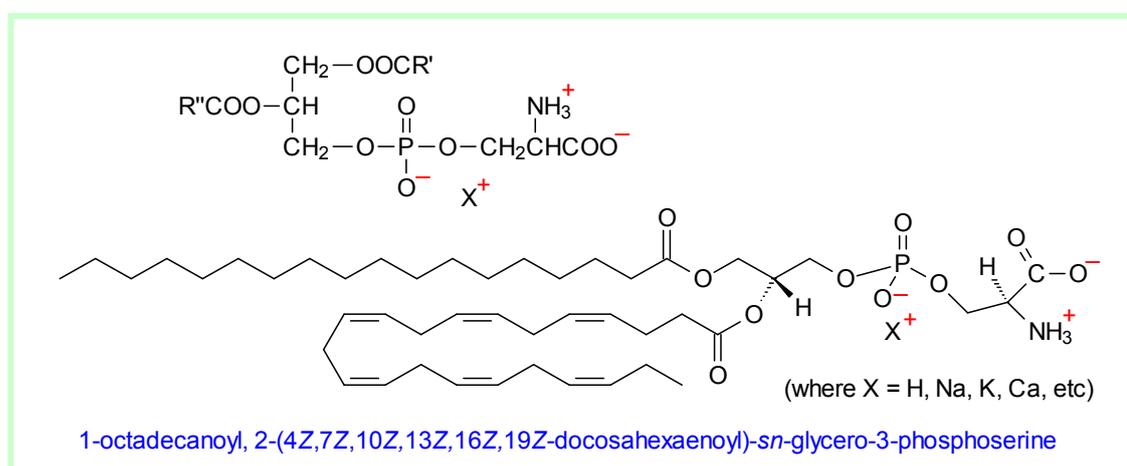


PHOSPHATIDYLSERINE AND RELATED LIPIDS

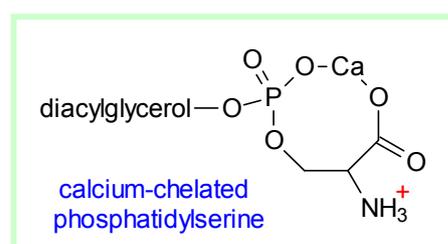
STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

1. Phosphatidylserine – Structure and Occurrence

Although **phosphatidylserine** or 1,2-diacyl-*sn*-glycero-3-phospho-L-serine is distributed widely among animals, plants and microorganisms, it is usually less than 10% of the total phospholipids, the greatest concentration being in myelin from brain tissue. However, it may comprise 10 to 20 mol% of the total phospholipid in the plasma membrane and endoplasmic reticulum of the cell. In yeasts, such as *S. cerevisiae*, it is a minor component of most other cellular organelles other than the plasma membrane, where it can amount to more than 30% of the total lipids. In most bacteria, it is a minor membrane lipid, although it is important as an intermediate in phosphatidylethanolamine biosynthesis. The 1-octadecanoyl-2-docosahexaenoyl molecular species, which may be especially important in brain tissue, is illustrated here.



Phosphatidylserine is an acidic (anionic) phospholipid with three ionizable groups, i.e. the phosphate moiety, the amino group and the carboxyl function. As with other acidic lipids, it exists in nature in salt form, but it has a high propensity to chelate to calcium via the charged oxygen atoms of both the carboxyl and phosphate moieties, modifying the conformation of the polar head group. This interaction may be of considerable relevance to the biological function of phosphatidylserine, especially during bone formation for example.



In animal cells, the fatty acid composition of phosphatidylserine varies from tissue to tissue, but does not appear to resemble the precursor phospholipids, either because of selective utilization of specific molecular species for biosynthesis or because of remodelling of the lipid via deacylation-reacylation reactions with lysophosphatidylserine as an intermediate (see below). In human plasma, 1-stearoyl-2-oleoyl and 1-stearoyl-2-arachidonoyl species predominate, but in brain (especially grey matter), retina and many other tissues 1-stearoyl-2-docosahexaenoyl species are very abundant. Indeed, the ratio of *n*-3 to *n*-6 fatty acids in brain phosphatidylserine is much higher than in most other lipids. The positional distribution of fatty acids in phosphatidylserine from rat liver and bovine brain are listed in **Table 1**. As with most phospholipids, saturated fatty acids are concentrated in position *sn*-1 and polyunsaturated in position *sn*-2.

Table 1. Positional distribution of fatty acids in phosphatidylserine from rat liver and bovine brain.

Position	Fatty acid					
	16:0	18:0	18:1	18:2	20:4	22:6
Rat liver [1]						
<i>sn</i> -1	5	93	1			
<i>sn</i> -2	6	29	8	4	32	19
Bovine brain [2]						
<i>sn</i> -1	3	81	13			
<i>sn</i> -2	2	1	25	trace	1	60

1. Wood, R. and Harlow, R.D. *Arch. Biochem. Biophys.*, **135**, 272-281 (1969).

2. Yabuuchi, H. and O'Brien, J.S. *J. Lipid Res.*, **9**, 65-67 (1968).

In leaves of *Arabidopsis thaliana*, used as a 'model' plant in many studies, the fatty acid composition of phosphatidylserine resembles that of phosphatidylethanolamine.

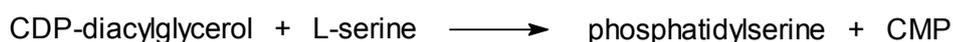
Phosphatidylserines with ether-linked moieties (alkyl and alkenyl) are not common in animal tissues. They were first found in rat lung, but they are reported to be relatively abundant in human lens and macrophages.

As a generality, the concentration of phosphatidylserine is highest in plasma membranes and endosomes, but is very low in mitochondria. As it is located entirely on the inner monolayer surface of the plasma membrane (and of other cellular membranes) and it is the most abundant anionic phospholipid, it may make the largest contribution to interfacial effects in membranes involving non-specific electrostatic interactions. This normal distribution is disturbed during platelet activation and cellular apoptosis.

2. Biosynthesis of Phosphatidylserine

L-Serine is a non-essential amino acid that is actively synthesised by most organisms. In animals, it is produced in nearly all cell types, although in brain it is synthesised by astrocytes but not by neurons, which must be supplied with this amino acid for the biosynthesis of phosphatidylserine (and of sphingoid bases).

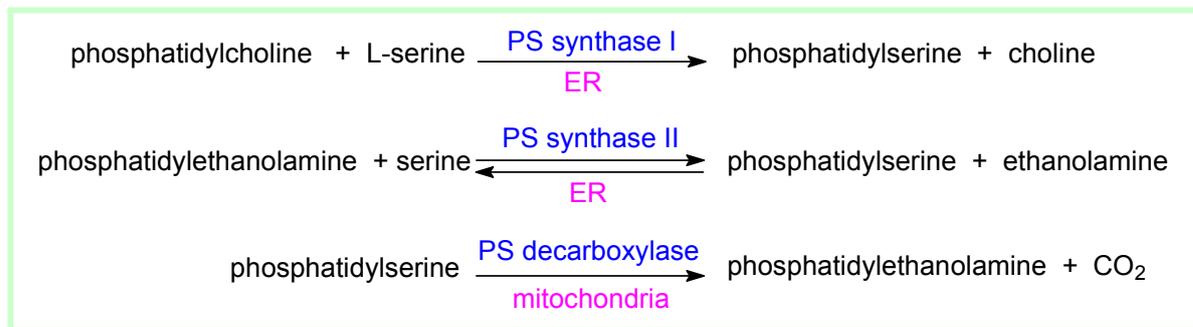
In bacteria and other prokaryotic organisms, phosphatidylserine is synthesised by a mechanism comparable to that of other phospholipids, i.e. by reaction of L-serine with CDP-diacylglycerol (see our web pages on **phosphatidylglycerol**).



Much of the phosphatidylserine thus formed is decarboxylated to phosphatidylethanolamine, and this may be the major route to the latter in bacteria. As phosphatidylcholine in yeast is produced via methylation of phosphatidylethanolamine, phosphatidylserine is the primary precursor phospholipid in these organisms.

In contrast in animal tissues, there are two routes to phosphatidylserine involving distinct enzymes (PS synthase I and II) with 30% homology and several membrane-spanning domains but utilizing

different substrates. Phosphatidylserine is synthesised in the endoplasmic reticulum of the cell, or in a specific domain of this termed the mitochondria-associated membrane, because it is tethered transiently to the mitochondrial outer membrane. The reaction involves exchange of L-serine with phosphatidylcholine, catalysed by PS synthase I, or with phosphatidylethanolamine, catalysed by PS synthase II. It is strictly dependent on calcium ions and requires no further source of energy. The new lipid is then transported to the mitochondria, probably by transient membrane contact, where it is decarboxylated to phosphatidylethanolamine by a specific decarboxylase. The latter can return to the endoplasmic reticulum where it may be converted back to phosphatidylserine by the action of PS synthase II.



Phosphatidylserine synthase-I is expressed in all mouse tissues, but especially the kidney, liver and brain, while phosphatidylserine synthase-II is most active in the brain and testis and much less so in other tissues. The latter enzyme has a high specificity for docosahexaenoic acid. It is not known why such a complex series of coupled reactions is necessary, or why there should be two enzymes. One virtue of the second enzyme is that the free ethanolamine and choline formed are rapidly re-utilized for phospholipid synthesis. Thus, both phosphatidylserine and phosphatidylethanolamine are produced without a reduction in the amount of phosphatidylcholine. Elimination of both enzymes is embryonically lethal in knock-out mice, but each of them can be knocked out separately and the mice survive, even though they have substantially reduced levels of phosphatidylserine and phosphatidylethanolamine. It is evident that cellular concentrations of these two lipids are intimately related and tightly regulated.

As with other phospholipids, the final fatty acid composition in animal tissues is attained by a process of remodelling known as the Lands' cycle (see the webpage on phosphatidylcholine, for example). The first step, is hydrolysis by a phospholipase A₂ to lysophosphatidylserine, followed by reacylation by various acyl-CoA:lysophospholipid acyltransferases. One membrane-bound O-acyltransferase (MBOAT1) with a preference for oleoyl-CoA has been characterized, while a second (MBOAT5 or LPCAT3) incorporates linoleoyl and arachidonoyl chains (and also utilizes lysophosphatidylcholine).

In plants, much of the phosphatidylserine appears to be produced by a calcium-dependent base-exchange reaction in which the head-group of an existing phospholipid is exchanged for L-serine (i.e. mechanistically similar to PS synthase 1), but a CDP-diacylglycerol pathway exists in some species, e.g. wheat.

N-Acylphosphatidylserine has been reported as a minor component of the lipids of sheep erythrocytes, bovine brain and the central nervous system of freshwater fish, amongst others. The *N*-arachidonoyl form may be the precursor of the endocannabinoid **N-arachidonoylserine**.

3. Phosphatidylserine – Biological Function

In addition to its function as a component of cellular membranes and as a precursor for other phospholipids, phosphatidylserine is an essential cofactor that binds to and activates a large

number of proteins, especially those with signalling activities. The presence of appreciable amounts of phosphatidylserine on the cytosolic leaflet of endosomes and lysosomes enables these compartments to dock with proteins with specific phosphatidylserine-binding domains, including several important signaling and fusogenic effectors. The cytoskeletal protein spectrin binds to phosphatidylserine, for example. In addition, the high concentration of this anionic lipid results in an accumulation of negative surface charge to which poly-cationic proteins can bind. The effect is believed to be that certain proteins are re-directed from one target membrane to another. It is also required by other enzymes, such as Na^+/K^+ ATPase and neutral sphingomyelinase. Phosphatidylserine is not involved in cell signalling through the formation of metabolites, as is the case with phosphatidylinositol.

Phosphatidylserine is involved in the blood coagulation process in platelets, where it is transported from the inner to the outer surface of membrane vesicles that are derived from activated platelets. Here, phosphatidylserine enhances the activation of prothrombin to thrombin (the key molecule in the blood clotting cascade) directly, or by binding to specific sites on two key regulatory factors. Apolipoprotein A-1 in high-density lipoproteins has a controlling function in that it neutralizes these procoagulant properties by arranging the phospholipid in surface areas that are too small to accommodate the prothrombinase complex.

Phosphatidylserine is known to have an important role in the regulation of apoptosis (programmed cell death) in response to particular calcium-dependent stimuli. The normal distribution of this lipid on the inner leaflet of the membrane bilayer is then disrupted because of stimulation of enzymes such as flippases or scramblase, which can move phosphatidylserine in both directions across the membrane, and inhibition of aminophospholipid translocases, which returns the lipid to the inner side of the membrane. After transfer to the outer leaflet of the cell, it is believed that a receptor on the surface of macrophages and related scavenger cells recognizes the phosphatidylserine and facilitates the removal of the apoptotic cells and their potentially toxic or immunogenic contents in a non-inflammatory manner. Binding of phosphatidylserine to specific proteins, such as apolipoprotein H (β 2-glycoprotein 1), enhances the recognition and clearance. This process is essential for the development of lung and brain, and it is also relevant to clinical situations where apoptosis plays an important part, such as cancer, chronic autoimmunity, and infections.

The process of apoptosis is often accompanied by generation of reactive oxygen species, which bring about rapid oxidation of the fatty acids in phosphatidylserine before this lipid is externalized. Indeed, it is now apparent that only molecular species of phosphatidylserine with an oxidatively truncated *sn*-2 acyl group that incorporates terminal γ -hydroxy(or oxo)- α,β -unsaturated acyl moieties are recognized by scavenger receptors in macrophages as a prerequisite for engulfment of apoptotic cells.

In addition, appreciable amounts of phosphatidylserine are translocated by a similar mechanism to the surface of T lymphocytes that express low levels of the trans-membrane enzyme tyrosine phosphatase. This change in distribution acts then as a signalling mechanism to modulate the activities of several membrane proteins. The protein annexin V binds with high specificity to phosphatidylserine and is used as a probe to detect apoptotic cells.

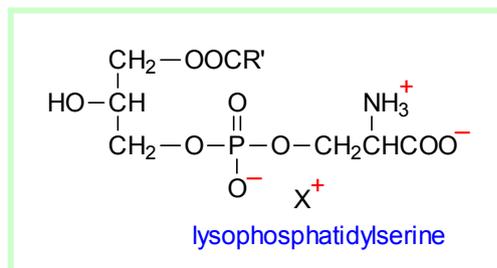
A further unusual function of phosphatidylserine is that it is a key component of the lipid-calcium-phosphate complexes that initiate mineral deposition during the formation of bone. It has been established that phosphatidylserine and inorganic phosphate must be present, before calcium ions are introduced, when the high affinity of phosphatidylserine for calcium ions becomes important.

The high concentrations of docosahexaenoic acid (DHA) in brain and retinal phosphatidylserine are certainly important for the development and function of these tissues. Accumulation of phosphatidylserine in neuronal membranes is promoted by DHA, and this is important for the maintenance of neuronal survival. Phosphatidylserine may also be a reservoir of DHA for **protectin** formation in neuronal tissue. On the other hand, the Food and Drug Administration in the USA

considers that there is little scientific evidence to support claims that dietary supplements of phosphatidylserine reduce the risk of dementia or cognitive dysfunction in the elderly. Other nutritional claims also appear doubtful. Antibodies to phosphatidylserine are formed in some disease states, including thrombosis and recurrent spontaneous pregnancy loss.

4. Lysophosphatidylserine

Lysophosphatidylserine, with a fatty acid in position *sn*-1 only, is known to be a mediator of a number of biological processes, and is presumably formed primarily by deacylation of phosphatidylserine by phospholipases. It has been detected after injury to animal tissues (tumor growth, graft rejection, burns), and it may have a similar function to **lysophosphatidic acid** in cell signalling, for example in regulating calcium flux through a specific receptor. There is also a mammalian phosphatidylserine-specific phospholipase A_1 , which hydrolyses the *sn*-1 acyl group to generate *sn*-2-lysophosphatidylserine.

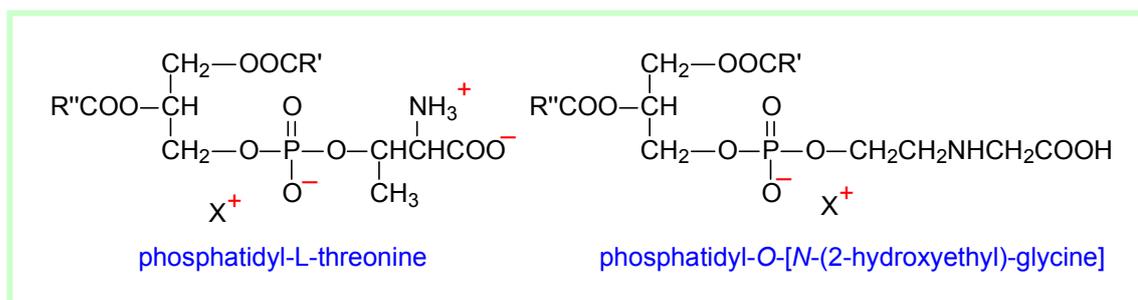


Lysophosphatidylserine can be generated when cells are damaged, when it can diffuse and transmit the information to other cells, especially mast cells, and it is produced to enhance clearance of activated and dying neutrophils. It also has a role in the resolution of inflammation. In Schistosome infections, lysophosphatidylserine from the parasite is believed to be a key activator molecule in the host. Quite specifically, the *sn*-2-lyso-phosphatidylserine stimulates degranulation of mast cells – most other lysophospholipids have no such activity.

In addition, negatively charged lysophosphatidylserine derivatives tend to organize in non-bilayer structures and are believed to facilitate folding of certain membrane proteins *in situ* better than bilayer-forming lipids.

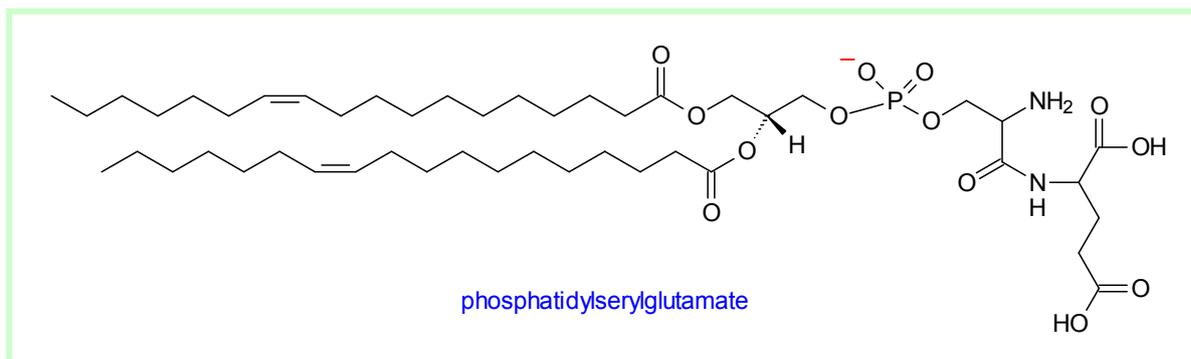
5. Phosphatidylthreonine and Other Amino Acid-Containing Phospholipids

Phosphatidyl-L-threonine, which is closely related structurally and metabolically to phosphatidylserine, was first detected in animal brain and tuna muscle, before it was characterized definitively as a minor component of polyoma virus-transformed embryo fibroblasts in hamsters, cultured hippocampal neurons and macrophages. It has also been detected in some bacterial species. Biosynthetic studies with microsomes from rat brain suggest that it is synthesised by the same base-exchange enzyme involved in phosphatidylserine synthesis but with much lower activity. In laboratory animals, it is barely detectable in normal tissues such as brain, and it is decarboxylated in mitochondria *in vitro* to phosphatidylisopropanolamine. Phosphatidyl-L-aspartate and phosphatidyl-L-glutamate with carboxylate-phosphate anhydride bonds have recently been detected in rat brain.



Lysophosphatidylthreonine, with a fatty acid in position *sn*-1 only, displays many of the biological activities reported for lysophosphatidylserine *in vitro*, although it is not known whether it is also active *in vivo*.

A further amino acid-linked phospholipid, phosphatidyl-O-[*N*-(2-hydroxyethyl)glycine] has been isolated from brown algae of the family Phaeophyceae, such as *Fucus serratus*, where it can amount to as much as 25% of the total lipids. The fatty acid composition is distinctive in that arachidonic acid comprises about 80% of the total. A minor phospholipid component from the bacterium *Escherichia coli* contains a dipeptide unit, i.e. phosphatidylserylglutamate.



Phosphatidylethanolamineglutamate has been detected in the bacterium *Peredibacter starrii*. Other amino acid-containing phospholipids (**complex lipoamino acids**) are more closely related to phosphatidylglycerol in structure and biosynthesis and are discussed elsewhere on this site.

6. Analysis

As with other acidic lipids, the metal ions associated with phosphatidylserine hamper analysis, although the problem can be solved by an acid wash. It is easily separated from other phospholipids by two-dimensional thin-layer chromatography, but poorly shaped peaks are often seen with high-performance liquid chromatography. Mass spectrometry is being used increasingly for molecular species analysis and quantification.

Recommended Reading

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