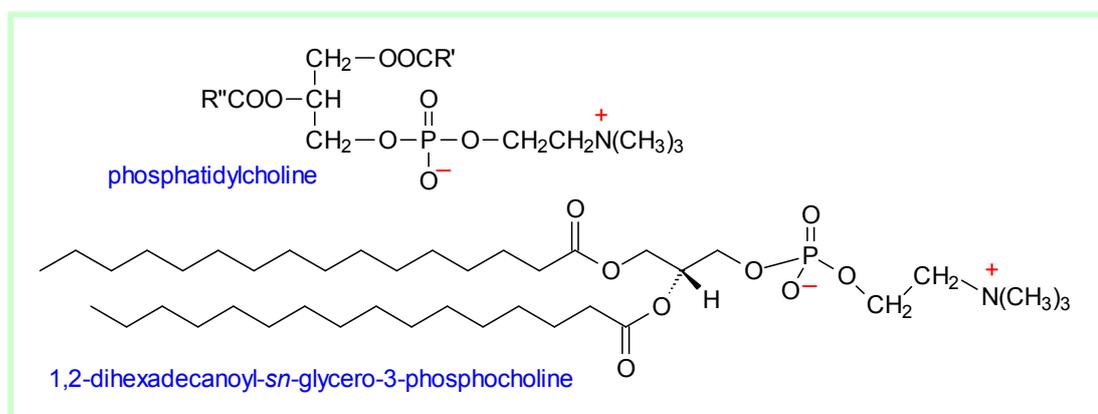


PHOSPHATIDYLCHOLINE AND RELATED LIPIDS

STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

1. Phosphatidylcholine – Structure and Occurrence

Phosphatidylcholine (once given the trivial name 'lecithin') is usually the most abundant phospholipid in animals and plants, often amounting to almost 50% of the total, and as such it is obviously a key building block of membrane bilayers. In particular, it makes up a very high proportion of the outer leaflet of the plasma membrane. Phosphatidylcholine is also the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL. On the other hand, it is less often found in bacterial membranes, perhaps 10% of species, but there is none in the 'model bacteria', *Escherichia coli* and *Bacillus subtilis*.



It is a neutral or zwitterionic phospholipid over a pH range from strongly acid to strongly alkaline. In animal tissues, some of its membrane functions appear to be shared with the structurally related sphingolipid, **sphingomyelin**, although the latter has many unique properties of its own.

In animal tissues, phosphatidylcholine tends to exist in mainly in the diacyl form, but small proportions (in comparison to phosphatidylethanolamine and phosphatidylserine) of alkylacyl and alkenylacyl forms may also be present. Data for the compositions of these various forms from bovine heart muscle are listed in our web pages on **ether lipids**.

As a generalization, animal phosphatidylcholine tends to contain lower proportions of arachidonic and docosahexaenoic acids and more of the C₁₈ unsaturated fatty acids than the other zwitterionic phospholipid, **phosphatidylethanolamine**. The saturated fatty acids are most abundant in position *sn*-1, while the polyunsaturated components are concentrated in position *sn*-2. Indeed, C₂₀ and C₂₂ polyenoic acids are almost exclusively in position *sn*-2. Dietary factors obviously influence fatty acid compositions, but in comparing animal species, it would be expected that the structure of the phosphatidylcholine in the same metabolically active tissue would be somewhat similar in terms of the relative distributions of fatty acids between the two positions. **Table 1** lists some representative data.

There are some exceptions to the rule as the phosphatidylcholine in some tissues or organelles contains relatively high proportions of disaturated molecular species. For example, it is well known that lung phosphatidylcholine in most if not all animal species studied to date contains a high proportion (50% or more) of dipalmitoyl-phosphatidylcholine.

Table 1. Positional distribution of fatty acids in the phosphatidylcholines of some animal tissues.

Position	Fatty acid						
	16:0	16:1	18:0	18:1	18:2	20:4	22:6
Rat liver [1]							
<i>sn-1</i>	23	1	65	7	1	trace	
<i>sn-2</i>	6	1	4	13	23	39	7
Rat heart [2]							
<i>sn-1</i>	30	2	47	9	11	-	-
<i>sn-2</i>	10	1	3	17	20	33	9
Rat lung [3]							
<i>sn-1</i>	72	4	15	7	3	-	-
<i>sn-2</i>	54	7	2	12	11	10	1
Human plasma [4]							
<i>sn-1</i>	59	2	24	7	4	trace	-
<i>sn-2</i>	3	1	1	26	32	18	5
Human erythrocytes [4]							
<i>sn-1</i>	66	1	22	7	2	-	-
<i>sn-2</i>	5	1	1	35	30	16	4
Bovine brain (gray matter) [5]							
<i>sn-1</i>	38	5	32	21	1	-	-
<i>sn-2</i>	33	4	trace	48	1	9	4
Chicken egg [6]							
<i>sn-1</i>	61	1	27	9	1	-	-
<i>sn-2</i>	2	1	trace	52	33	7	4

1, Wood, R. and Harlow, R.D. *Arch. Biochem. Biophys.*, **131**, 495-501 (1969); 2, Kuksis, A. et al. *J. Lipid Res.*, **10**, 25-32 (1969); 3, Kuksis, A. et al. *Can. J. Physiol. Pharm.*, **46**, 511-524 (1968); 4, Marai, L. and Kuksis, A. *J. Lipid Res.*, **10**, 141-152 (1969); 5, Yabuuchi, H. and O'Brien, J.S. *J. Lipid Res.*, **9**, 65-67 (1968); 6, Kuksis, A. and Marai, L. *Lipids*, **2**, 217-224 (1967).

The positional distributions of fatty acids in phosphatidylcholine in representative plants and yeast are listed in **Table 2**. In the leaves of the model plant *Arabidopsis thaliana*, saturated fatty acids are concentrated in position *sn-1*, but monoenoic fatty acids are distributed approximately equally between the two positions, and there is a preponderance of di- and triunsaturated fatty acids in position *sn-2*. The same is true for soybean 'lecithin'. The pattern is somewhat similar for the yeast *Lipomyces lipoferus*, except that much of the 16:1 is in position *sn-1* in this instance.

Table 2. Composition of fatty acids (mol %) in positions *sn*-1 and *sn*-2 in the phosphatidylcholine from plants and yeast.

Position	Fatty acid					
	16:0	16:1	18:0	18:1	18:2	18:3
<i>A. thaliana</i> (leaves) [1]						
<i>sn</i>-1	42	trace	4	5	23	26
<i>sn</i>-2	1	trace	trace	5	47	47
Soybean 'lecithin' [2]						
<i>sn</i>-1	24		9	14	47	4
<i>sn</i>-2	5		1	13	75	6
<i>Lipomyces lipoferus</i> [3]						
<i>sn</i>-1	24	18	trace	37	16	4
<i>sn</i>-2	4	5	trace	39	31	19

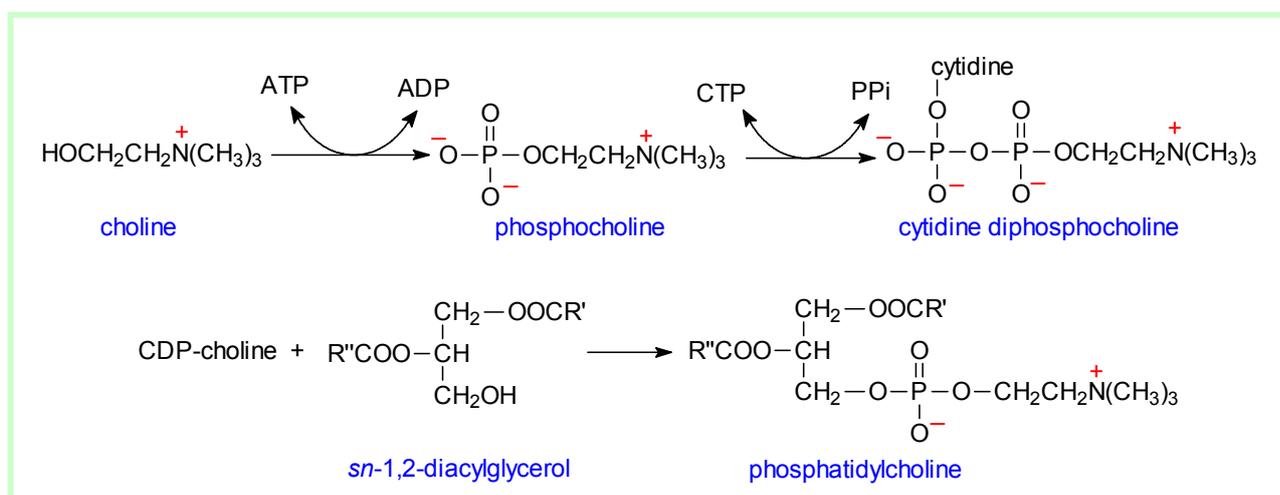
1, Browse, J., Warwick, N., Somerville, C.R. and Slack, C.R. *Biochem. J.*, **235**, 25-31 (1986).

2, Blank, M.L., Nutter, L.J. and Privett, O.S. *Lipids*, **1**, 132-135 (1966).

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2. Phosphatidylcholine – Biosynthesis

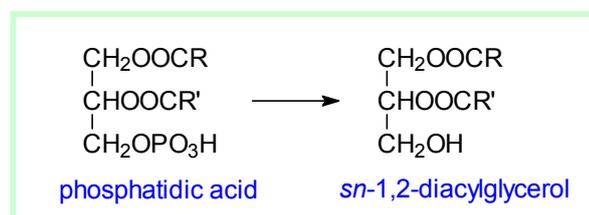
There are several mechanisms for the biosynthesis of phosphatidylcholine in animals, plants and micro-organisms. Choline itself is not synthesised as such by animal cells and is an essential nutrient. It must be obtained from dietary sources or by degradation of existing choline-containing lipids, for example those produced by the second pathway described below. Once taken up into cells, choline is immediately phosphorylated by a choline kinase in the cytoplasm of the cell to phosphocholine, which is reacted with cytidine triphosphate (CTP) to form cytidine diphosphocholine. The membrane-bound enzyme CDP-choline:1,2-diacylglycerol choline-phosphotransferase in the endoplasmic reticulum catalyses the reaction of the last compound with *sn*-1,2-diacylglycerol to form phosphatidylcholine. This is the main pathway for the synthesis of phosphatidylcholine in animals and plants, and it is analogous to the biosynthesis of **phosphatidylethanolamine** (see our web pages on this lipid).



The discovery of the importance of this pathway depended a little on serendipity in that in experiments in the lab of Professor Eugene Kennedy, samples of adenosine triphosphate (ATP) contained some cytidine triphosphate (CTP) as an impurity. However, luck is of little value without receptive minds, and Kennedy and co-workers demonstrated that the impurity was an important metabolite that was essential for the formation of phosphatidylcholine.

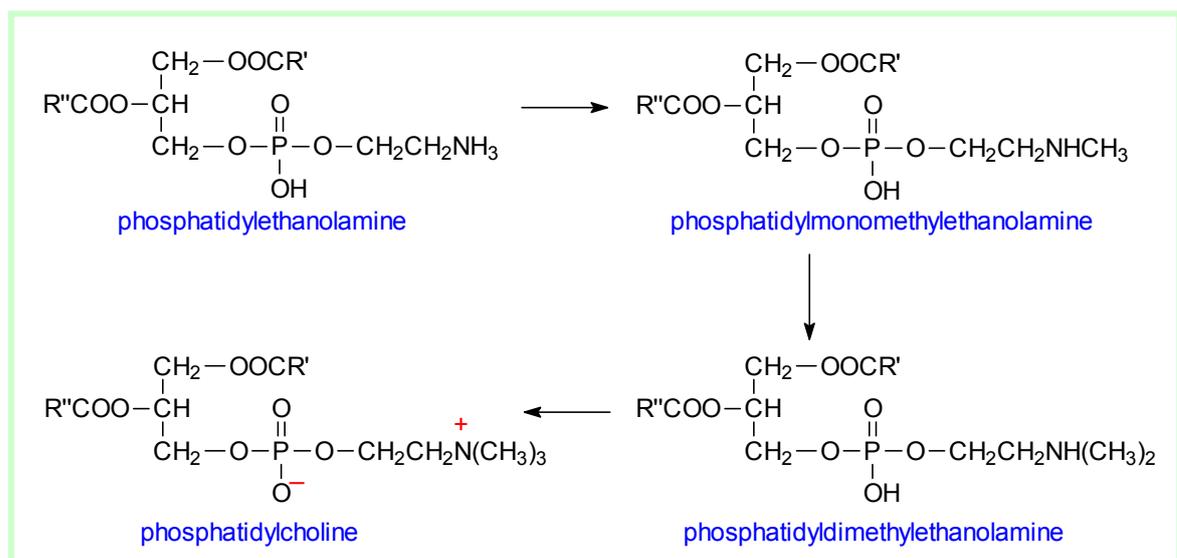
The above reaction, together with the biosynthetic mechanism for phosphatidylethanolamine, is significantly different from that for phosphatidylglycerol, phosphatidylinositol and cardiolipin. Both make use of nucleotides, but with the latter, the nucleotide is covalently linked directly to the lipid intermediate, i.e. **cytidine diphosphate diacylglycerol**. However, a comparable pathway for biosynthesis of phosphatidylcholine occurs in bacteria (see below).

The source of the *sn*-1,2-diacylglycerol precursor, which is also a key intermediate in the formation of phosphatidylethanolamine and phosphatidylserine, and of triacylglycerols, is **phosphatidic acid**. In this instance, the important enzyme is phosphatidic acid phosphatase (or 'phosphatidate phosphatase' or 'lipid phosphate phosphatase' or 'phosphatidate phosphohydrolase').



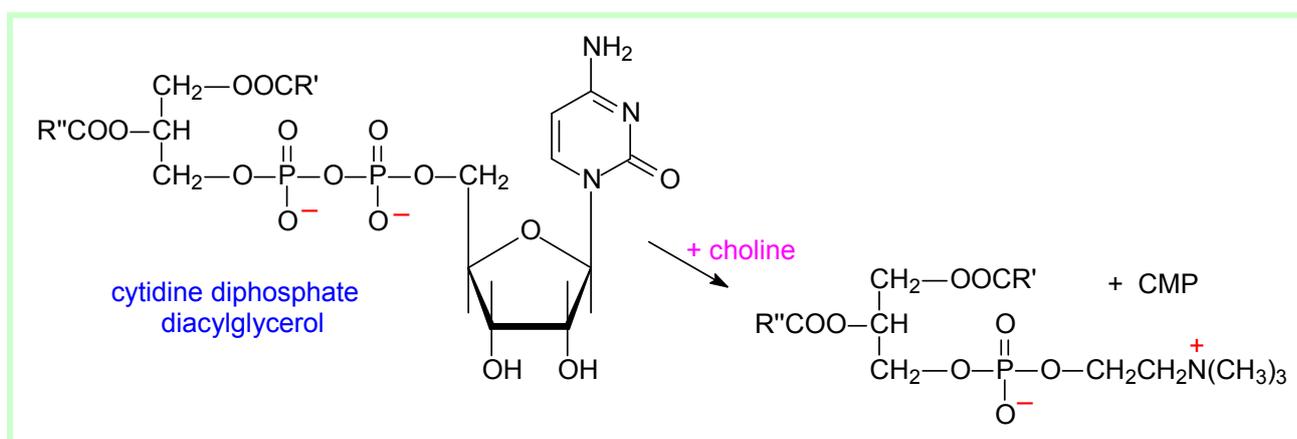
This enzyme is also important for the production of diacylglycerols as essential intermediates in the biosynthesis of triacylglycerols and of **phosphatidylethanolamine**. Yeasts contain two such enzymes, Mg^{2+} dependent (PAP1) and Mg^{2+} independent (PAP2). In mammals, much of the phosphatidic acid phosphatase activity resides in three related cytoplasmic proteins, termed lipin-1, -2, and -3 (see our webpage on **triacylglycerol** biosynthesis). Lipin-1 is found mainly in adipose tissue, while lipin-2 is present mainly in liver.

The second pathway for biosynthesis of phosphatidylcholine involves sequential methylation of phosphatidylethanolamine, with *S*-adenosylmethionine as the source of methyl groups, with **mono- and dimethyl-phosphatidylethanolamine** (see the web pages) as intermediates and catalysed by the enzyme phosphatidylethanolamine *N*-methyltransferase. A single enzyme (~20 Kda) catalyses all three reactions and is located mainly in the endoplasmic reticulum where it spans the membrane.



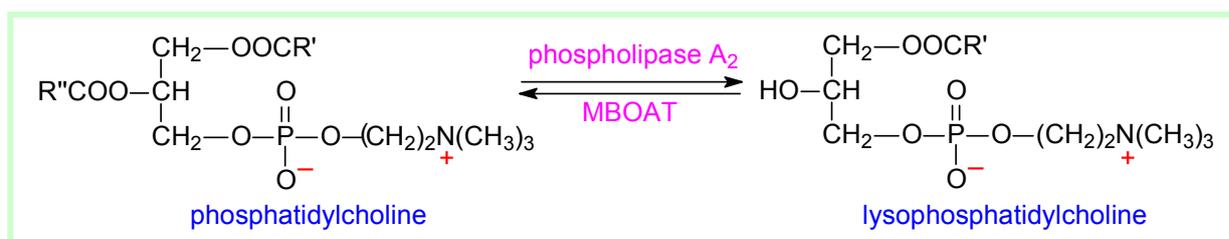
This is a major pathway in the liver, but not in other animal tissues or in general in higher organisms. It may be the main route to phosphatidylcholine in those bacterial species that produce this lipid and in yeasts, but it does not appear to operate in higher plants. This liver pathway is especially important when choline is deficient in the diet. Phosphatidylcholine biosynthesis by both pathways in the liver is necessary for normal secretion of the plasma lipoproteins (VLDL and HDL), and it is relevant to a number of human physiological conditions. A by-product of the biosynthesis of phosphatidylcholine from phosphatidylethanolamine is the conversion of S-adenosylmethionine to S-adenosylhomocysteine, which is hydrolysed in the liver to adenosine and homocysteine. An elevated level of the latter in plasma is a risk factor for cardiovascular disease and myocardial infarction.

A third pathway for phosphatidylcholine biosynthesis was found first in one bacterial species symbiotic with plants (*Sinorhizobium meliloti*), though it is now known to occur more widely. In this instance, the lipid is formed in one step via condensation of choline directly with CDP-diacylglycerol, with cytidine monophosphate (CMP) formed as a byproduct; the choline comes from the host plant. In *Agrobacterium* species and some other bacteria, both this route and that via phosphatidylethanolamine operate.



The yeast *Saccharomyces cerevisiae*, is able to reacylate endogenously generated glycerophosphocholine with acyl-CoA in the microsomal membranes, first to lysophosphatidylcholine and then to phosphatidylcholine. While phosphatidylcholine is a major lipid in yeasts, recent work suggests that it is not essential if suitable alternative growth substrates are available, unlike higher organisms where perturbation of phosphatidylcholine synthesis can lead to inhibition of growth or even cell death. Enhanced synthesis of phosphatidylcholine appears to occur in cancer cells and solid tumours, and this may prove to be a target for therapeutic agents.

Whatever the mechanism of biosynthesis in tissues, it is apparent that the fatty acid compositions and positional distributions on the glycerol moiety are determined post synthesis by extensive re-modelling involving hydrolysis (phospholipase A₂ mainly) and re-acylation, a process that is sometimes termed the '**Lands Cycle**' after its discoverer W.E.M. (Bill) Lands.



There are at least fifteen different groups of enzymes in the phospholipase A₂ super-family, which differ in calcium dependence, cellular location, and structure. All hydrolyse the *sn*-2 ester bond of

phospholipids specifically, generating a fatty acid and lysophospholipid, both of which have important functions in their own right in addition to their role in the Lands cycle. There is also a phospholipase A₁, which is able to cleave the *sn*-1 ester bond.

The re-acylation step is catalysed by a membrane-bound coenzyme A-dependent lysophosphatidylcholine acyltransferase (MBOAT5 also designated 'LPCAT3'), which has been located chiefly within the endoplasmic reticulum, though also in mitochondria and the plasma membrane, in organs such as the liver, adipose tissue and pancreas. This enzyme incorporates linoleoyl and arachidonoyl chains specifically into lysophosphatidylcholine (see below). Polyunsaturated fatty acids introduced by this route can then be transferred to 1-alkyl and 1-alkenyl phospholipids by CoA-independent transacylases. A second such enzyme LPCAT4 is known and has a clear preference for 18:1-CoA. Similarly, the highly saturated molecular species of phosphatidylcholine found in the nucleus are formed from species with a more conventional composition by remodelling, presumably by acyltransferases with somewhat different specificities. These and further related enzymes are involved in remodelling of all other phospholipids.

It should be noted that all of these pathways for the biosynthesis of diacyl-phosphatidylcholine are very different and are separated spatially from that producing **alkyl-acyl- and alkenyl-acyl-phosphatidylcholines** *de novo* (see our webpage on **ether lipids**). Also, synthesis of phosphatidylcholine does not occur uniformly throughout the endoplasmic reticulum but is located at membrane interfaces or where it meets other organelles, and especially where the membrane is expanding dynamically.



In plants, fatty acids esterified to phosphatidylcholine can serve as substrates for desaturases, and this means that the fatty acid composition changes after the initial synthetic process here also. The process is further complicated in plants in that biosynthesis or partial synthesis (via lysophosphatidylcholine) occurs in different organelles, such as the endoplasmic reticulum, plastids and mitochondria, from different fatty acid pools or with differing specificities.

On **catabolism** of choline-containing lipids, much of the choline is re-used for phosphatidylcholine biosynthesis, often after being returned to the liver (the CDP-choline cycle). Some is oxidized in the kidney and liver to betaine, which serves as a donor of methyl groups for S-adenosylmethionine production. A proportion is used in nervous tissues for production of acetylcholine, which is a neurotransmitter of importance to learning, memory and sleep. Some choline is lost through excretion of phosphatidylcholine in bile.

3. Phosphatidylcholine – Biological Functions

Because of the generally cylindrical shape of the molecule, phosphatidylcholine spontaneously organizes into bilayers, so it is ideally suited to serve as the bulk structural element of biological membranes, and as outlined above it makes up a high proportion of the lipids in the outer leaflet of the plasma membrane. The unsaturated acyl chains are kinked and confer fluidity on the membrane. Such properties are essential to act as a balance to those lipids that do not form bilayers or that form specific microdomains such as **rafts**. While phosphatidylcholine does not induce curvature of membranes, as may be required for membrane transport and fusion processes, it can be metabolized to form lipids that do.

In contrast, dipalmitoyl phosphatidylcholine is the main surface-active component of human lung surfactant, although in other animals the lung surfactant can be enriched in some combination of short-chain disaturated and monounsaturated species, mainly palmitoylmyristoyl- and palmitoylpalmitoleoyl- in addition to the dipalmitoyl-lipid. This is believed to provide alveolar stability by decreasing the surface tension at the alveolar surface to a very low level. Also, the internal lipids of the animal cell nucleus (after the external membrane has been removed) contain a high

proportion of disaturated phosphatidylcholine, amounting to 10% of the volume indeed. This is synthesised entirely within the nucleus, unlike phosphatidylinositol for example, and in contrast to other cellular lipids its composition cannot be changed by extreme dietary manipulation. It has been suggested that it may have a role in stabilizing or regulating the structure of the chromatin, as well as being a source of diacylglycerols with a signalling function.

As noted above, phosphatidylcholine is by far the most abundant phospholipid component in plasma and indeed in all plasma **lipoprotein** classes. It is the only phospholipid necessary for lipoprotein assembly and secretion. Although it is especially abundant in high density lipoproteins (HDL), it influences strongly the levels of all circulating lipoprotein classes and especially of the very-low-density lipoproteins (VLDL), which are surrounded by a phospholipid monolayer. Similarly, phosphatidylcholine synthesis is required to stabilize the surface of lipid droplets in tissues where **triacylglycerols** are stored.

In addition to its function as a membrane and lipoprotein constituent, phosphatidylcholine may have a role in signalling via the generation of **diacylglycerols** (see our web page on this lipid) by phospholipase C, especially in the nucleus. Although the pool of the precursor is so great in many tissues that turnover is not easily measured, the presence of phospholipases C and D specific for phosphatidylcholine, which are activated by a number of agonists, suggests such a function especially in the cell nucleus. Diacylglycerols formed in this way would be much more saturated than those derived from phosphatidylinositol, and would not be expected to be as active. Diacylglycerols formed by the action of a family of enzymes of the phospholipase C type may be more important in plants, especially during phosphate deprivation, to generate precursors for galactolipid biosynthesis and perhaps lipid re-modelling more generally. Similarly, **phosphatidic acid** generated from phosphatidylcholine by the action of phospholipase D in plants has key signalling functions.

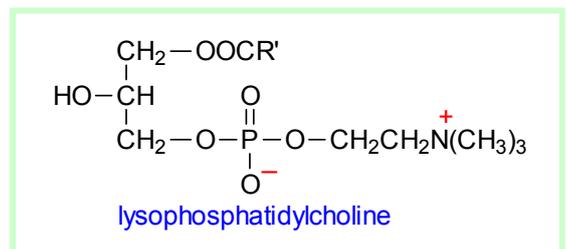
The plasmalogen form of phosphatidylcholine may also have a signalling function, as thrombin treatment of endothelial cells activates a selective hydrolysis (phospholipase A₂) of molecular species containing arachidonic acid in the *sn*-2 position, releasing this fatty acid for eicosanoid production. The diacyl form of phosphatidylcholine may have a related function in signal transduction in other tissues. In addition, it is known that the enzyme 3-hydroxybutyrate dehydrogenase requires to be bound to phosphatidylcholine before it can function optimally.

Phosphatidylcholine is the biosynthetic precursor of **sphingomyelin** and as such must have some influence on the many metabolic pathways that constitute the sphingomyelin cycle. It is also a precursor for phosphatidic acid, lysophosphatidylcholine and platelet-activating factor, each with important signalling functions, and of phosphatidylserine.

In prokaryotes, phosphatidylcholine is essential for certain symbiotic and pathogenic microbe-host interactions. For example, in human pathogens such as *Brucella abortus* and *Legionella pneumophila*, this lipid is necessary for full virulence, and the same is true for plant pathogens, such as *Agrobacterium tumefaciens*. Bacteria symbiotic with plants, e.g. the rhizobial bacterium *Bradyrhizobium japonicum*, require it to establish efficient symbiosis and root nodule formation.

4. Lysophosphatidylcholine

Lysophosphatidylcholine, with one mole of fatty acid per mole of lipid in position *sn*-1, is found in trace amounts in most tissues (at greater concentrations, it disrupts membranes). It is produced by hydrolysis of dietary and biliary phosphatidylcholine and is absorbed as such in the intestines, but it is re-esterified before being exported in the lymph. In addition, it is formed in



most tissues by hydrolysis of phosphatidylcholine by means of the superfamily of phospholipase A₂ enzymes as part of the de-acylation/re-acylation cycle that controls the overall molecular species composition of the latter, as discussed above. In plasma of animal species, an appreciable amount of lysophosphatidylcholine is formed by the action of the enzyme lecithin:cholesterol acyltransferase (LCAT), which is secreted from the liver. This catalyses the transfer of fatty acids from position *sn*-2 of phosphatidylcholine to free cholesterol in plasma, with formation of **cholesterol esters** and of course of lysophosphatidylcholine (see our webpage on **lipoproteins**), which consists of a mixture of molecular species with predominately saturated and mono- and dienoic fatty acid constituents. In plasma, it is bound to albumin and lipoproteins so that its effective concentration is reduced to a safe level. Identification of a highly specific phospholipase A₂ in peroxisomes that generates 2-arachidonoyl lysophosphatidylcholine suggests that this may be of relevance to eicosanoid generation and signalling.

Lysophosphatidylcholine has pro-inflammatory properties and it is known to be a pathological component of oxidized lipoproteins (LDL) in plasma and of atherosclerotic lesions; it has been shown to promote demyelination in the nervous system. Recently, it has been found to have some functions in cell signalling, and specific receptors (coupled to G proteins) have been identified. It activates the specific phospholipase C that releases diacylglycerols and inositol triphosphate with resultant increases in intracellular Ca²⁺ and activation of protein kinase C. It also activates the mitogen-activated protein kinase in certain cell types. In vascular endothelial cells, it induces the important pro-inflammatory mediator cyclooxygenase 2 (COX-2), a key enzyme in **prostaglandin** synthesis. Some biological effects of lysophosphatidylcholine may be simply due to its ability to diffuse readily into membranes, altering their curvature and indirectly affecting the properties of membrane proteins.

In contrast, stearoyl lysophosphatidylcholine has an anti-inflammatory role in that it is protective against lethal sepsis in experimental animals by various mechanisms, including stimulation of neutrophils to eliminate invading pathogens through a peroxide-dependent reaction. Similarly, there are suggestions that lysophosphatidylcholine may have beneficial effects in rheumatoid arthritis.

Amylose-rich starch granules of cereal grains contain lysophosphatidylcholine as virtually the only lipid in the form of inclusion complexes or lining channels in the macromolecules.

5. Other Phosphatidylcholine Analogues

Phosphatidylarsenocholine is a minor component of the lipids of a number of marine organisms and is discussed in the web page dealing with arsenolipids. Similarly, **phosphatidylsulfocholine** is described on the web page dealing with sulfonolipids.

Platelet-activating factor (PAF) or 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is an ether analogue of phosphatidylcholine that is biologically active. Because it is so distinctive, it is discussed in its own web page.

6. Analysis

Analysis of phosphatidylcholine presents no particular problems. It is readily isolated by thin-layer or high-performance liquid chromatography methods. Determination of the dipalmitoyl species in lung surfactant is a more demanding task, but specific methods have been published. Phospholipase A₂ from snake venom is used in methods to determine the position of fatty acids on the glycerol moiety. Modern mass spectrometry methodology has greatly simplified the task of molecular species analysis. Lysophosphatidylcholine can be formed inadvertently and over-estimated as a consequence of careless extraction of lipids from tissues.

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