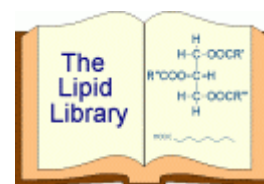# PHOSPHATIDYLCHOLINE AND RELATED LIPIDS

STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

1. Phosphatidylcholine – Structure and Occurrence

Phosphatidylcholine (once given the trivial name 'lecithin') is usually the most abundant phospholipid in animal and plants, often amounting to almost 50% of the total, and as such it is obviously the 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. 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.

phosphatidylcholine

1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine
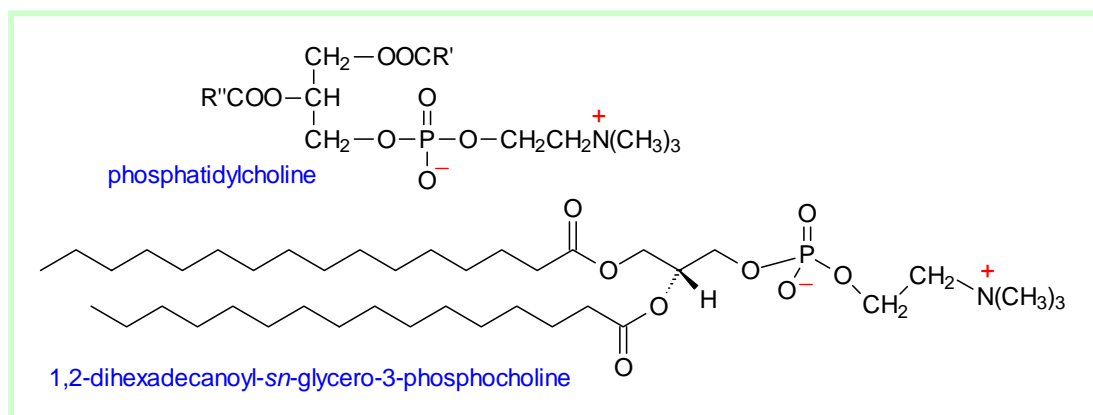
In animal tissues, phosphatidylcholine tends to exist in mainly in the diacyl form, but small proportions (in comparison to phosphatidylethanolamine and phosphatidylserine) of alkylacyl and alkenylacyl forms may also be present. Data for the compositions of these various forms from bovine heart muscle 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 C_{18} 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, C_{20} and C_{22} polyenoic acids are 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. The phosphatidylcholine in some organs 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. It appears that this is the main surface-active component, providing alveolar stability by decreasing the surface tension at the alveolar surface to

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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.

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

Position	16:0	16:1	18:0	Fatty acid 18:1	18:2	20:4	22:6
Rat liver [1]							
sn-1	23	1	65	7	1	trace	
sn-2	6	1	4	13	23	39	7
Rat heart [2]							
sn-1	30	2	47	9	11	-	-
sn-2	10	1	3	17	20	33	9
Rat lung [3]							
sn-1	72	4	15	7	3	-	-
sn-2	54	7	2	12	11	10	1
Human plasma [4]							
sn-1	59	2	24	7	4	trace	-
sn-2	3	1	1	26	32	18	5
Human erythrocytes [4]							
sn-1	66	1	22	7	2	-	-
sn-2	5	1	1	35	30	16	4
Bovine brain (gray matter) [5]							
sn-1	38	5	32	21	1	-	-
sn-2	33	4	trace	48	1	9	4
Chicken egg [6]							
sn-1	61	1	27	9	1	-	-
sn-2	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
A. thaliana (leaves) [1]						
sn-1	42	trace	4	5	23	26
sn-2	1	trace	trace	5	47	47
Soybean 'lecithin' [2]						
sn-1	24		9	14	47	4
sn-2	5		1	13	75	6
L. lipoferus [3]						
sn-1	24	18	trace	37	16	4
sn-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).
3, Haley, J.E. and Jack, R.C. *Lipids*, **9**, 679-681 (1974).

2. Phosphatidylcholine – Biosynthesis and Biological Function

There are three mechanisms for the biosynthesis of phosphatidylcholine. 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 cholinephosphotransferase 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).
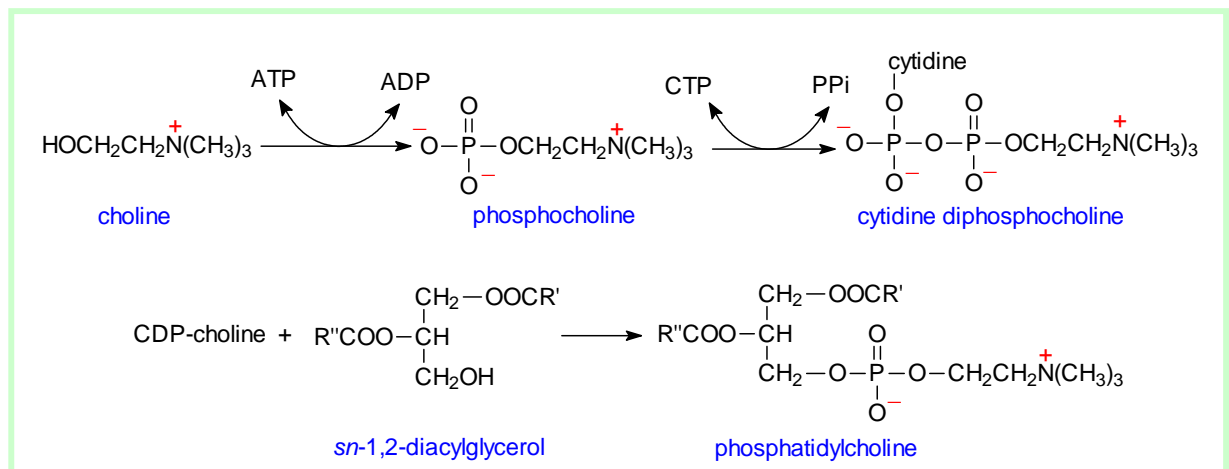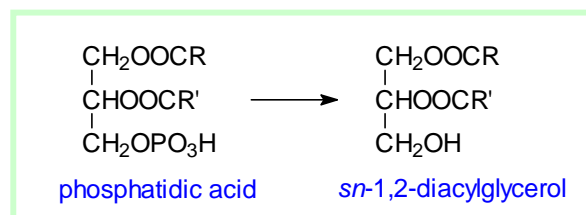

The discovery of the importance of this pathway depended a little on serendipity in that in experiments in the lab of Professor Eugene Kennedy, samples of adenosine triphosphate (ATP) contained some cytidine triphosphate (CTP) as an impurity. However, luck is of little value without

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**.

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, which is present mainly in the endoplasmic reticulum of the cell.

$$
\begin{array}{c}
CH_2OOCR \\
| \\
CHOOCR' \\
| \\
CH_2OPO_3H
\end{array}
\longrightarrow
\begin{array}{c}
CH_2OOCR \\
| \\
CHOOCR' \\
| \\
CH_2OH
\end{array}
$$

phosphatidic acid   *sn*-1,2-diacylglycerol

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.

phosphatidylethanolamine → phosphatidylmonomethylethanolamine → phosphatidyldimethylethanolamine → phosphatidylcholine
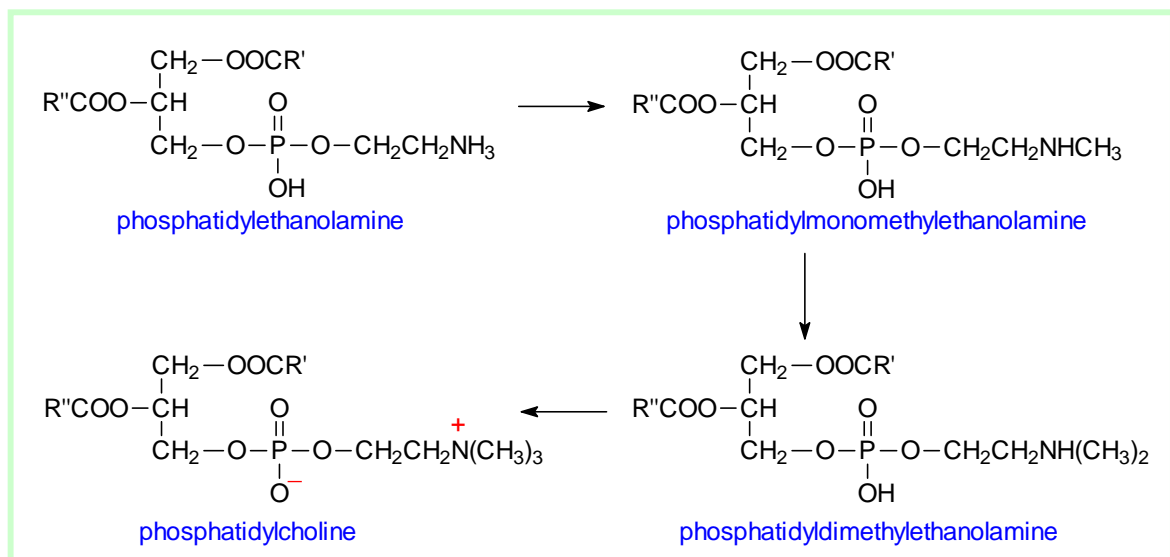
This liver enzyme is especially important when choline is deficient in the diet. A by-product of the biosynthesis of phosphatidylcholine from phosphatidylethanolamine is the conversion of S-adenosylmethionine to *S*-adenosylhomocysteine, which is hydrolysed in the liver to adenosine and homocysteine. It is noteworthy that elevated plasma homocysteine is a risk factor for cardiovascular disease and myocardial infarction.

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.

In one bacterial species, a third pathway for phosphatidylcholine biosynthesis that is choline-dependent has been found in which the lipid is formed in one step via condensation of choline directly with CDP-diacylglycerol.

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 (phospholipases A_1, A_2 or B) and re-acylation, a process that is sometimes termed the 'Lands cycle'. The re-acylation step is catalysed by a specific lysophosphatidylcholine acyltransferase, which has been located within the endoplasmic reticulum in organs such as the liver, adipose tissue and pancreas. For example, the highly saturated molecular species of phosphatidylcholine found in the nucleus are formed from species with a more conventional composition by remodelling. In plants, fatty acids esterified to phosphatidylcholine can serve as substrates for desaturases, and this means that the fatty acid composition changes also after the initial synthetic process. 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.

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. 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**.

In addition to its function as a membrane constituent, phosphatidylcholine may have a role in signalling via the generation of diacylglycerols, 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. The plasmalogen form of phosphatidylcholine may also have a signalling function, as thrombin treatment of endothelial cells activates a selective hydrolysis (phospholipase A_2) 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.

On catabolism of choline-containing lipids, much of the choline is re-used for phosphatidylcholine biosynthesis, often after being returned to the liver. 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 the bile.

3. Lysophosphatidylcholine

Lysophosphatidylcholine, with one mole of fatty acid per mole of lipid in position *sn*-1, is found in small amounts in most tissues. It is formed by hydrolysis of phosphatidylcholine by the enzyme phospholipase A_2, as part of the de-acylation/re-acylation cycle that controls its overall molecular species composition. It can also be formed inadvertently during extraction of lipids from tissues if the phospholipase is activated by careless handling. There is also a phospholipase A_1, which is able to cleave the *sn*-1 ester bond.

lysophosphatidylcholine
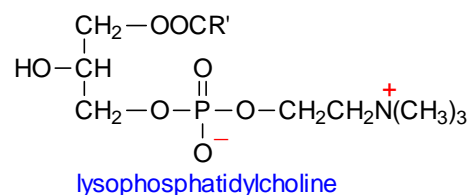
In plasma of animal species, appreciable amounts of lysophosphatidylcholine are formed by a specific enzyme system, lecithin:cholesterol acyltransferase (LCAT), which is secreted from the liver. The enzyme catalyses the transfer of the fatty acids of position *sn*-2 of phosphatidylcholine to the free cholesterol in plasma, with formation of **cholesterol esters** and of course of lysophosphatidylcholine. Identification of a highly specific phospholipase A_2 in peroxisomes that generates 2-arachidonoyl lysophosphatidylcholine suggests that this may be of relevance to eicosanoid generation and signalling.

Lysophosphatidylcholine has also been shown to be a pathological component of oxidized lipoproteins (LDL) in plasma and of atherosclerotic lesions. 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 Ca^{2+} and activation of protein kinase C. It also activates the mitogen-activated protein kinase in certain cell types.

Stearoyl lysophosphatidylcholine has been shown to be protective against lethal sepsis in experimental animals by various mechanisms, including stimulation of neutrophils to eliminate invading pathogens through a peroxide-dependent reaction.

4. Platelet-Activating Factor

Platelet-activating factor (PAF) or 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is an ether analogue of phosphatidylcholine that is biologically active. For convenience, it is discussed in our web pages on **Ether lipids**.

5. 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 A_2 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.

Recommended Reading

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o   Veldhuizen, R., Nag, K., Orgeig, S. and Possmayer, F. The role of lipids in pulmonary surfactant. *Biochim. Biophys. Acta*, **1408**, 90-108 (1998).

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