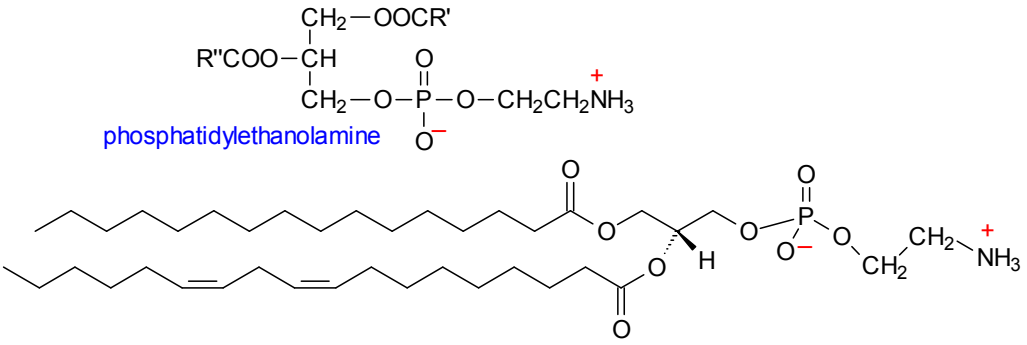
PHOSPHATIDYLETHANOLAMINE AND RELATED LIPIDS

STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

1. Phosphatidylethanolamine – Structure and Occurrence

Phosphatidylethanolamine (once given the trivial name 'cephalin') is usually the second most abundant phospholipid in animal and plant lipids and it is frequently the main lipid component of microbial membranes. It can amount to 20% of liver phospholipids and as much as 45% of those of brain; higher proportions are found in mitochondria than in other organelles. As such, it is obviously a key building block of membrane bilayers. It is a neutral or zwitterionic phospholipid (at least in the pH range 2 to 7) with the structure shown (with one specific molecular species illustrated as an example).



In animal tissues, phosphatidylethanolamine tends to exist in diacyl, alkylacyl and alkenylacyl forms, and data for the compositions of these various forms from bovine heart muscle are listed in our web pages on **ether lipids**. As much as 70% of the phosphatidylethanolamine in some cell types (especially inflammatory cells, neurons and tumor cells) can have an ether linkage.

Table 1. Positional distribution of fatty acids in phosphatidylethanolamine in animal tissues.

Position	Fatty acid						
	14:0	16:0	18:0	18:1	18:2	20:4	22:6
Rat liver [1]							
***sn*-1**		25	65	8			
***sn*-2**	2	11	8	8	10	46	13
Chicken egg [2]							
***sn*-1**		32	59	7	1		
***sn*-2**		1	1	25	22	29	12

1, Wood, R. and Harlow, R.D., *Arch. Biochem. Biophys.*, **131**, 495-501 (1969).
2, Holub, B.J. and Kuksis, A. *Lipids*, **4**, 466-472 (1969).

In general, animal phosphatidylethanolamine tends to contain higher proportions of arachidonic and docosahexaenoic acids than the other zwitterionic phospholipid, phosphatidylcholine. These

polyunsaturated components are concentrated in position *sn*-2 with saturated fatty acids most abundant in position *sn*-1, as illustrated for rat liver and chicken egg in **Table 1**. In most other species, it would be expected that the structure of the phosphatidylethanolamine in the same metabolically active tissues would exhibit similar features.

The positional distributions of fatty acids in phosphatidylethanolamine from the leaves of the model plant *Arabidopsis thaliana* are listed in **Table 2**. Here also saturated fatty acids are concentrated in position *sn*-1, and there is a preponderance of di- and triunsaturated in position *sn*-2. The pattern is somewhat different for the yeast *Lipomyces lipoferus*, where the differences between the two positions are relatively minor.

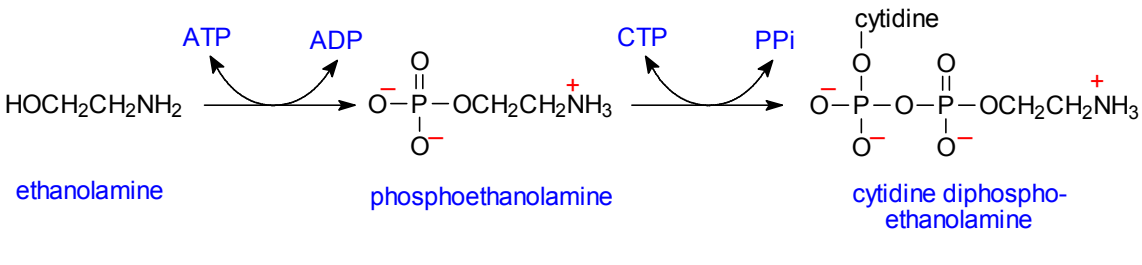
Table 2. Composition of fatty acids (mol %) in positions *sn*-1 and *sn*-2 in the phosphatidylethanolamine from leaves of *Arabidopsis thaliana* [1] and from *Lipomyces lipoferus* [2].

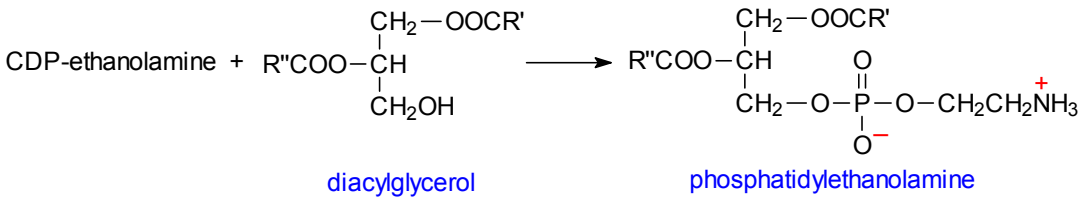
Position	Fatty acid					
	16:0	16:1	18:0	18:1	18:2	18:3
A. thaliana						
***sn*-1**	58	trace	4	5	15	18
***sn*-2**	trace	trace	trace	2	60	38
L. lipoferus						
***sn*-1**	29	18	4	28	13	6
***sn*-2**	23	15	3	34	17	6

1, Browse, J., Warwick, N., Somerville, C.R. and Slack, C.R. *Biochem. J.*, **235**, 25-31 (1986).
2, Haley, J.E. and Jack, R.C. *Lipids*, **9**, 679-681 (1974).

2. Phosphatidylethanolamine – Biosynthesis

A major pathway for biosynthesis of phosphatidylethanolamine *de novo* in animals and plants follows one of the general routes to phospholipid biosynthesis -

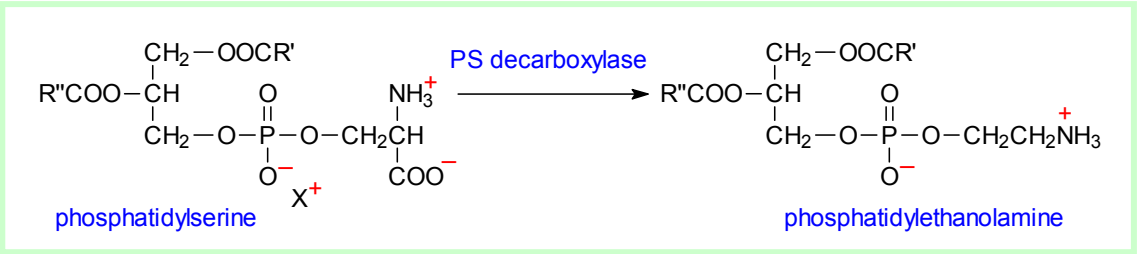




Ethanolamine is obtained by decarboxylation of serine in plants, and in animals most must come from dietary sources (a small amount comes from sphingolipid catabolism via **sphingosine-1-phosphate**). The first step in phosphatidylethanolamine biosynthesis is phosphorylation of

ethanolamine by the cytosolic enzyme ethanolamine kinase, followed by the rate-limiting step, i.e. reaction of the product with cytidine triphosphate (CTP) to form cytidine diphosphoethanolamine. In the final step, a membrane-bound enzyme in the endoplasmic reticulum CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase, catalyses the reaction of the last compound with diacylglycerol to form phosphatidylethanolamine. The diacylglycerol precursors are formed from phosphatidic acid via the action of the enzyme phosphatidic acid phosphohydrolase (see our web pages on **triacylglycerols** and **phosphatidylcholine**).

At least four other minor pathways exist, of which the most important is the conversion of phosphatidylserine to phosphatidylethanolamine (as discussed also in our web pages on **phosphatidylserine**). In prokaryotic cells, such as *E. coli*, in which phosphatidylethanolamine is the most abundant membrane phospholipid, all of it is derived from phosphatidylserine decarboxylation. However, this can also be a major pathway in mammalian cells and yeasts, where phosphatidylserine decarboxylase is located on the external aspect of the mitochondrial inner membrane (yeasts have a second related enzyme in the Golgi). The reaction is regulated by the transport of newly synthesised phosphatidylserine from the endoplasmic reticulum to the mitochondria.



Studies with mammalian cell types *in vitro* suggest that the CDP-ethanolamine pathway preferentially produces molecular species with mono- or di-unsaturated fatty acids on the *sn*-2 position, while the phosphatidylserine decarboxylation reaction generates species with polyunsaturated fatty acids on the *sn*-2 position mainly.

The relative importance of these two main pathways for phosphatidylethanolamine synthesis in mammalian cells appears to depend on the cell type. Both are essential and for example, disruption of the phosphatidylserine decarboxylase gene causes misshapen mitochondria and has lethal consequences in embryonic mice. It is evident that cellular concentrations of phosphatidylethanolamine and phosphatidylserine are intimately related and tightly regulated.

Phosphatidylethanolamine can also be formed by the enzymatic exchange reaction of ethanolamine with phosphatidylserine, or by re-acylation of lysophosphatidylethanolamine. The last pathway is associated with the mitochondria-associated membrane where the phosphatidylserine synthase II is located. The bacterial plant pathogen *Xanthomonas campestris* is able to synthesise phosphatidylethanolamine by condensation of cytidine diphosphate diacylglycerol with ethanolamine. It should be noted that all of these pathways for the biosynthesis of diacyl-phosphatidylethanolamine are very different and are separated spatially from that producing **alkyl-acyl- and alkenyl-acyl-phosphatidylethanolamine** (see our webpage on **ether lipids**). In the protozoon *Trypanosoma brucei*, for example, it has been demonstrated that the diacyl and ether pools of phosphatidylethanolamine have separate functions and cannot substitute for each other.

Each of the four mechanisms forms different pools of phosphatidylethanolamine species, which are often in different cellular compartments and have distinctive compositions. 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 A_2 to lysophosphatidylglycerol, followed by reacylation by means of various acyl-CoA:lysophospholipid acyltransferases. At least two enzymes of this type specific for phosphatidylethanolamine have been characterized, while the enzymes

LPCAT3 and 4, which are involved in phosphatidylcholine biosynthesis, are also active with phosphatidylethanolamine. Some of these isoforms appear to be confined to particular tissues.

3. Phosphatidylethanolamine – Biological Function

Phosphatidylethanolamine is a precursor for the synthesis **N-acyl-phosphatidylethanolamine** (see below) and thence of **anandamide** (N-arachidonoylethanolamine), and it is the donor of ethanolamine phosphate during the synthesis of the **glycosylphosphatidylinositol anchors** that attach many signalling proteins to the surface of the plasma membrane. In bacteria, it functions similarly in the biosynthesis of lipid A and other **lipopolysaccharides**. It is also the substrate for the hepatic enzyme phosphatidylethanolamine *N*-methyltransferase, which provides about a third of the **phosphatidylcholine** in liver.

Although phosphatidylethanolamine has sometimes been equated with phosphatidylcholine in biological systems, there are significant differences in the chemistry and physical properties of these lipids, and they have different functions in biochemical processes. Both are key components of membrane bilayers. However, phosphatidylethanolamine has a smaller head group, which gives the lipid a cone shape, and it can hydrogen bond to proteins through its ionizable amine group. On its own, it does not form bilayers but inverted hexagonal phases. With other lipids in a bilayer, it is believed to exert a lateral pressure that modulates membrane curvature and stabilizes membrane proteins in their optimum conformation. In contrast to phosphatidylcholine, it is concentrated with phosphatidylserine in the inner leaflet of the plasma membrane. It appears that a primary role for phosphatidylethanolamine in bacterial membranes at least is simply to dilute the high negative charge density of the anionic phospholipids.

Membrane proteins amount to 30% of the genome, and they carry out innumerable biochemical functions, including transport, energy production, biosynthesis, signalling and communication. Within a membrane, most integral proteins consist of hydrophobic α-helical trans-membrane domains that zigzag across it and are connected by hydrophilic loops. Of those parts of the proteins outwith the bilayer, positively charged residues are much more abundant on the cytoplasmic side of membrane proteins as compared to the trans side (the positive-inside rule). Phosphatidylethanolamine is believed to have a key function in that it inhibits location of negative amino acids on the cytoplasmic side, supporting the positive-inside rule, and it has an appropriate charge density to balance that of the membrane surface and the protein. However, it can also permit the presence of negatively charged residues on the cytosolic surface in some circumstances in support of protein function.

Much of the evidence for the unique properties of phosphatidylethanolamine comes from studies of the biochemistry of *E. coli*, where this lipid is a major component of the membranes. In particular, phosphatidylethanolamine has a specific involvement in supporting active transport by the lactose permease, and other transport systems may require or be stimulated by it. There is evidence that phosphatidylethanolamine acts as a ‘chaperone’ during the assembly of this and other membrane proteins to guide the folding path for the proteins and to aid in the transition from the cytoplasmic to the membrane environment. In the absence of this lipid, the transport membranes may not have the correct tertiary structure and so will not function correctly. Whether the lipid is required once the protein is correctly assembled is not fully understood in all cases, but it may be needed to orient enzymes correctly in the inner membrane. Phosphatidylethanolamine is certainly required both for proper functioning and to ensure the correct folding of the enzyme lactose permease (from *E. coli*) in membranes, although in contrast it inhibits folding of some multi-helical proteins. It appears that life in this organism can be maintained without phosphatidylethanolamine, but that life processes may be inhibited.

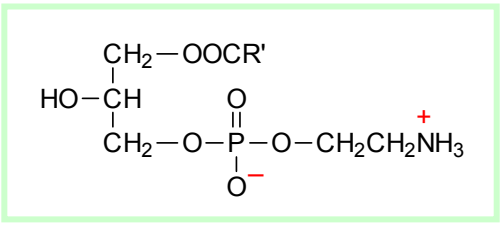
Although the mechanism has yet to fully elucidated, effects on protein conformation are believed to be behind a finding that phosphatidylethanolamine is the primary factor in brain required for the propagation and infectivity of mammalian prions.

In animal tissues, phosphatidylethanolamine is especially important in the sarcolemmal membranes of the heart during ischemia, it is involved in secretion of the nascent very-low-density lipoproteins from liver and it has functions in membrane fusion and fission. It has a functional role in the Ca^{2+}-ATPase in that one molecule of phosphatidylethanolamine is bound in a cavity between two transmembrane helices, acting as a wedge to keep them apart. This is displaced when Ca^{2+} is bound to the enzyme. A covalent conjugate of phosphatidylethanolamine with a protein designated 'Atg8' is involved in the process of autophagy (controlled degradation of cellular components) by promoting the formation of membrane vesicles containing the components to be degraded. Similarly, phosphatidylethanolamine is the precursor of an ethanolamine glycerophosphate moiety bound to two conserved glutamate residues in eukaryotic elongation factor 1A, which is an essential component in protein synthesis.

In the seeds of higher plants, a deficiency of phosphorylethanolamine cytidylyltransferase, a rate-limiting enzyme in the biosynthesis of phosphatidylethanolamine, has profound effects upon the viability and maturation of embryos.

4. Lysophosphatidylethanolamine

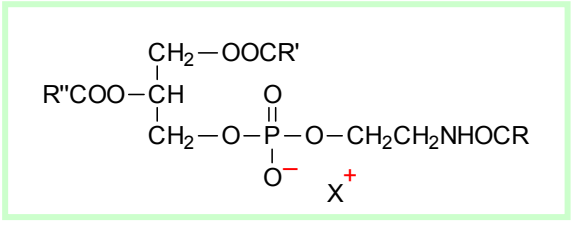
Lysophosphatidylethanolamine, with one mole of fatty acid per mole of lipid, is found in small amounts only in tissues. It is formed by hydrolysis of phosphatidylethanolamine by the enzyme phospholipase A_2, as part of a de-acylation/re-acylation cycle that controls its overall molecular species composition. A membrane-bound *O*-acyltransferase (MBOAT2) specific for lysophosphatidylethanolamine (and lysophosphatidic acid) has been characterized with a preference for oleoyl-CoA as substrate.



In plants, lysophosphatidylethanolamine is a specific inhibitor of phospholipase D, a key enzyme in the degradation of membrane phospholipids during the early stages of plant senescence. By this action, it retards the senescence of leaves, flowers, and post-harvest fruits. Indeed, it has a number of horticultural applications when applied externally, e.g. to stimulate ripening and extend the shelf-life of fruit, delay senescence and increase the vase life of cut flowers. In bacteria, lysophosphatidylethanolamine displays chaperone-like properties, promoting the functional folding of citrate synthase and other enzymes. Some biological properties have been reported in animal tissues *in vitro*, but a specific receptor has yet to be identified.

5. *N*-Acyl Phosphatidylethanolamine

In ***N*-Acyl phosphatidylethanolamine,** the free amino group of phosphatidylethanolamine is acylated by a further fatty acid. This lipid has been detected in rather small amounts in several animal tissues (~0.01%), but especially brain, nervous tissues and the epidermis, when the *N*-acyl chain is often palmitic or stearic acid (human plasma: *N*16:0-PE (40%), *N*18:1-PE (23.3%), *N*18:0-PE (19%), *N*18:2-PE (16.6%) and *N*20:4-PE (1.4%)). Under conditions of degenerative stress, it can accumulate in significant amounts, for example as the result of ischemic injury, infarction or cancer. It is present in plasma after feeding a high fat diet to rats, and that it can cross into the brain where it accumulates in the hypothalamus.



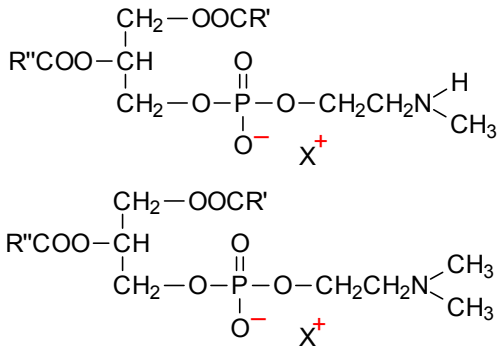
In animals, *N*-acyl phosphatidylethanolamine is formed biosynthetically by the action of a transferase exchanging a fatty acid from the *sn*-1 position of a phospholipid (probably phosphatidylcholine) to the primary amine group of phosphatidylethanolamine (without a hydrolytic step). In addition, some transfer can also occur from phosphatidylethanolamine *per se* by an intramolecular reaction. However, it should be noted that it can also be formed artefactually as a result of faulty extraction procedures during analysis. *N*-Acyl phosphatidylethanolamine is the precursor of **anandamide** and other biologically important amides in brain and other tissues (see our web pages on amides for a detailed discussion of *N*-acyl phosphatidylethanolamine synthesis and metabolism). It may have a separate role in regulating food intake, but this appears to be controversial.

In plants, *N*-acyl phosphatidylethanolamine is a common constituent of cereal grains (*e.g*. wheat, barley and oats) and of some other seeds (1.9% of the phospholipids of cotton seeds but 10-12% of oats). In other plant tissues, it is detected most often under conditions of physiological stress. It has also been found in a number of microbial species. Its synthesis involves direct acylation of phosphatidylethanolamine by coenzyme A esters, catalysed by a membrane-bound transferase. Activation of *N*-acyl phosphatidylethanolamine metabolism in plants with release of *N*-acylethanolamines seems to be associated with cellular stresses, but research is at an early stage.

N-Acetyl phosphatidylethanolamine was found in a filamentous fungus, *Absidia corymbifera*, where it comprised 6% of the total membrane lipids. It was accompanied by an even more unusual lipid 1,2-diacyl-*sn*-glycero-3-phospho(*N*-ethoxycarbonyl-ethanolamine.

6. Mono- and Dimethylphosphatidylethanolamines

Mono- and dimethylphosphatidylethanolamines are formed by sequential methylation of phosphatidyl-ethanolamine by the enzyme phosphatidyl-ethanolamine *N*-methyltransferase as intermediates in one mechanism for the biosynthesis of phosphatidylcholine. This is a minor pathway in general in animals, although it is significant in liver. However, it is the major route in yeasts and bacteria. although these lipids do not seem to be essential components of yeast membranes.

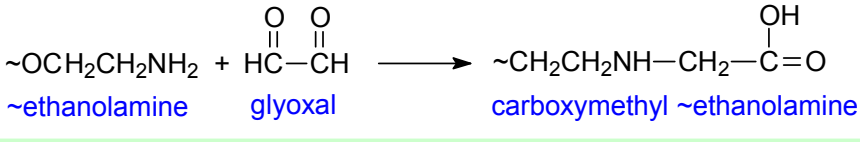


They are never found at greater than trace levels in animal or plant tissues, and it is not known whether they have any more specific functions. On the other hand as might be expected, they are more abundant in some species of bacteria, especially those that interact with plants.

7. Non-Enzymatic Modification of Phosphatidylethanolamine by Carbonyl-Amine Reactions

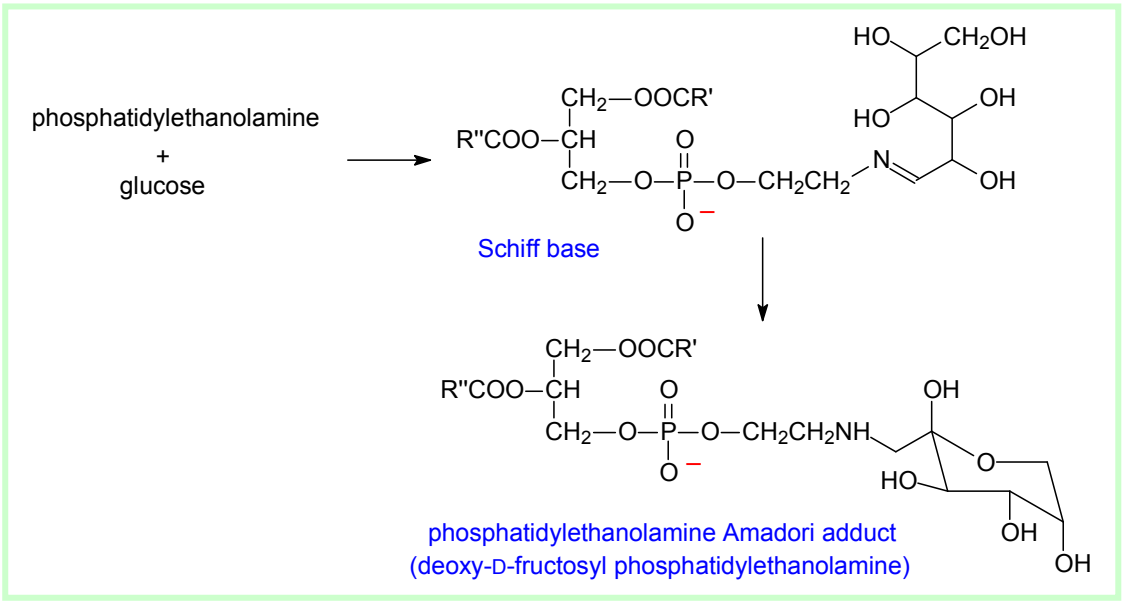
Phosphatidylethanolamine can react non-enzymatically to form Michael adducts with the hydroxy-alkenals and related compounds that are products of hydroperoxidation of unsaturated fatty acids, including malondialdehyde, acrolein, epoxyalkenals, hydroxyalkenals, oxoalkenals, and γ-keto-aldehydes. As an example the reaction with glycoxal is illustrated.

Such products accelerate the peroxidation of membrane lipids and are believed to be important for generating oxidative stress both in foods and in tissues. They are considered to be inflammatory mediators *in vivo* and have been implicated in a number of disease states, such as atherogenesis and diabetes, and during aging.

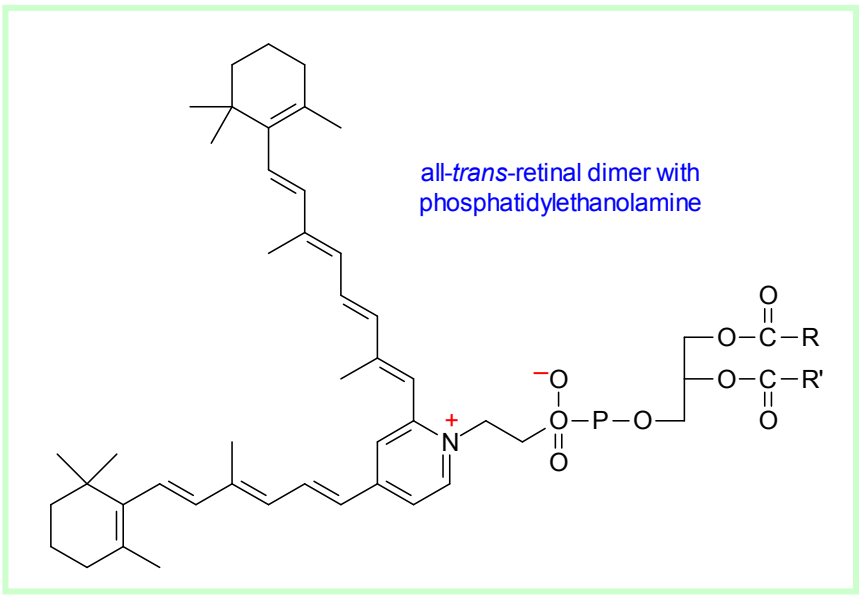


Similarly, levuglandins and isolevuglandins are reactive cyclo-oxygenase metabolites of arachidonic and docosahexaenoic acids. They react rapidly with the free amine group of phosphatidylethanolamine (and with proteins) *in vivo* to form cytotoxic hydroxylactam derivatives.

In recent years, the concept of the Maillard reaction has been expanded to include glycation of amino-phospholipids. For example, phosphatidylethanolamine reacts with glucose and other sugars to form first unstable Schiff bases, which rearrange to produce Amadori products of phosphatidylethanolamine, as illustrated for glucose below.



Once Amadori-phosphatidylethanolamine is formed, it can further undergo further reactions, for example to form carboxymethyl- and carboxyethyl-adducts, which also have the potential to trigger pathological processes. There are suggestions that Amadori-phosphatidylethanolamine may be a useful predictive marker for hyperglycemia in the early stages of diabetes especially. Phosphatidylserine might be expected to form similar materials, but these have proved harder to detect in tissues.

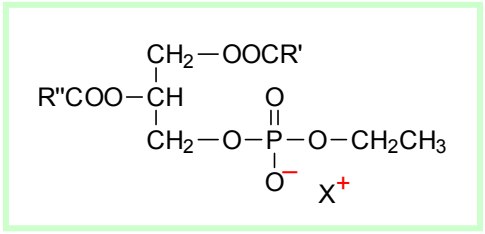


Phosphatidylethanolamine reacts with all-*trans*-retinal in the photoreceptor outer segment membrane of the eye to form first retinylidene-phosphatidylethanolamine, as part of a transport mechanism, but then a troublesome *bis*-retinoid condensation product.

This lipid conjugate together with hydrolysis products, formed by cleavage of the ethanolamine-phosphate bond, can accumulate in retinal pigment epithelial cells with age, and it may be involved in the pathogenesis of some retinal disorders.

8. Phosphatidylethanol

Phosphatidylethanol has little in common with phosphatidylethanolamine other than the obvious structural similarity. It is formed slowly in cell membranes, especially erythrocytes, by a transphosphatidylation reaction from phosphatidylcholine in the presence of ethanol, and catalysed by the enzyme phospholipase D. As such, it is a useful biochemical marker for alcohol abuse, since chronic alcoholics have very much higher levels in the blood than healthy subjects who consume alcohol in moderation.



9. Analysis

Analysis of phosphatidylethanolamine and related lipids present no particular problems. They are readily isolated by thin-layer or high-performance liquid chromatography methods for further analysis. Modern mass spectrometric methods are being used increasingly for the purpose.

Suggested Reading

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