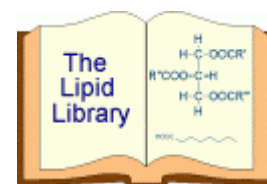


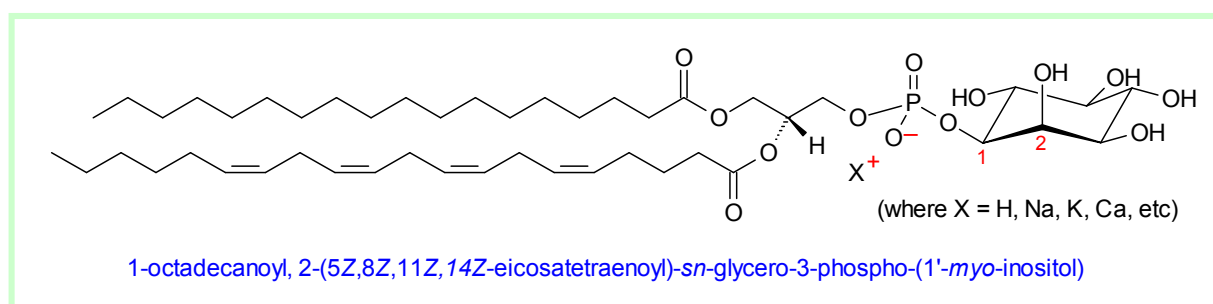
PHOSPHATIDYLINOSITOL AND RELATED LIPIDS

STRUCTURE, OCCURRENCE, COMPOSITION AND ANALYSIS



1. Phosphatidylinositol

Phosphatidylinositol is an important lipid, both as a key membrane constituent and as a participant in essential metabolic processes in all plants and animals, both directly and via a number of metabolites. It is an acidic (anionic) phospholipid that in essence consists of a phosphatidic acid backbone, linked via the phosphate group to inositol (hexahydroxycyclohexane). In most organisms, the stereochemical form of the latter is *myo*-D-inositol (with one axial hydroxyl in position 2 with the remainder equatorial), although other forms (*scyllo*- and *chiro*) have been found on occasion in plants. The 1-stearoyl,2-arachidonoyl molecular species, which is of considerable biological importance, is illustrated.



Phosphatidylinositol is especially abundant in brain tissue, where it can amount to 10% of the phospholipids, but it is present in all tissues and cell types. In rat liver, it amounts to 1.7 micromoles/g. There is usually less of it than of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

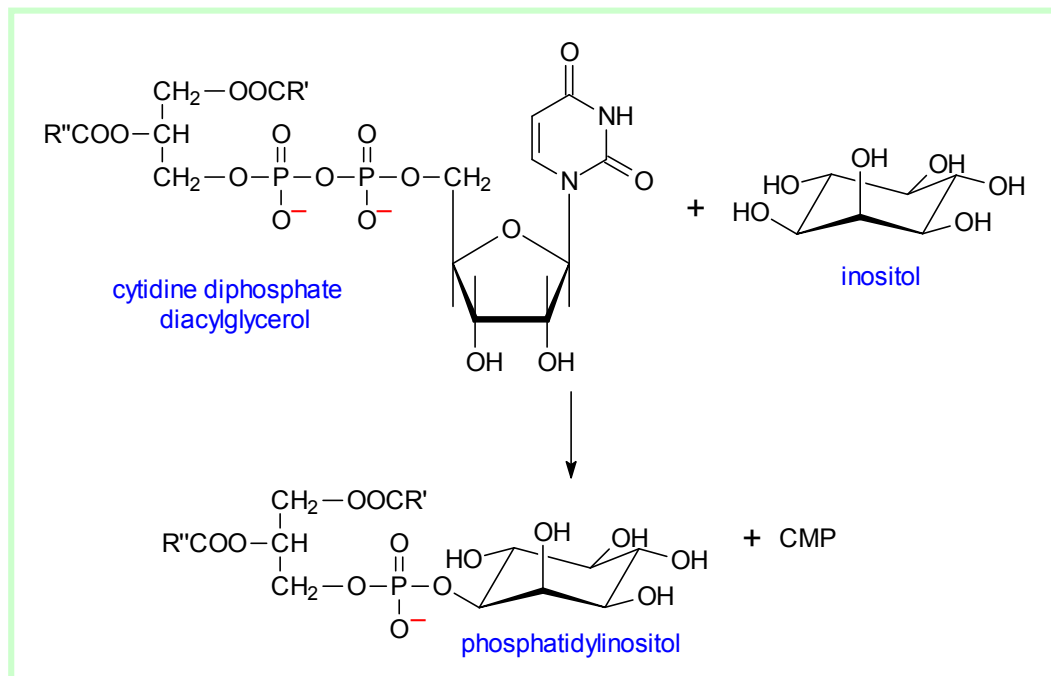
Table 1. Fatty acid composition of phosphatidylinositol (wt % of the total) in animal and plant tissues.

Tissue	Fatty acids									
	16:0	18:0	18:1	18:2	18:3	20:3	20:4	22:3	22:5	22:6
Bovine heart [1]	8	40	14	1	1	1	31	1	1	2
Bovine liver [2]	5	32	12	6	1	7	23	4	3	5
Rat liver [3]	5	49	2	2		4	35			1
<i>Arabidopsis thaliana</i> [4]	48	3	2	24	24					

[1] = Thompson,W. and MacDonald,G., *Eur. J. Biochem.*, **65**, 107-111 (1976). [2] = Thompson,W. and MacDonald,G., *J. Biol. Chem.*, **250**, 6779-6785 (1975). [3] = Wood,R. and Harlow,R.D. *Arch. Biochem. Biophys.*, **135**, 272-281 (1969). [4] = Browse,J., Warwick,N., Somerville,C.R. and Slack,C.R. *Biochem. J.*, **235**, 25-31 (1986).

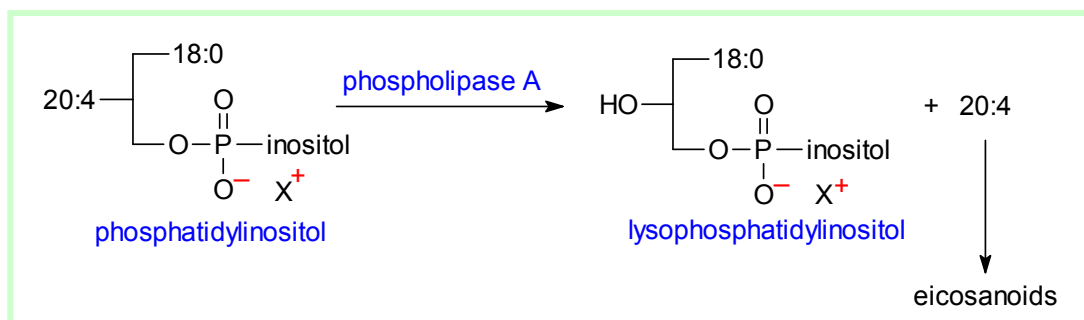
The fatty acid composition of phosphatidylinositol is rather distinctive as shown in **Table 1**. Thus, in animal tissues, the characteristic feature is a high content of stearic and arachidonic acids. All the stearic acid is linked to position *sn*-1 and all the arachidonic acid to position *sn*-2, and as much as 78% of the total lipid may consist of the single molecular species *sn*-1-stearoyl-*sn*-2-arachidonoylglycerophosphorylinositol (see **Table 2** below). Although 1-alkyl- and alkenyl- forms of phosphatidylinositol are known, they tend to be much less abundant than the diacyl form. In plant phosphatidylinositol, palmitic acid is the main saturated fatty acid while linoleic and linolenic acids are the main unsaturated components. Again, much of the saturated fatty acids are in position *sn*-1 and the unsaturated in position *sn*-2.

As with **phosphatidylglycerol** (and thence cardiolipin), phosphatidylinositol is formed biosynthetically from the precursor **cytidine diphosphate diacylglycerol** by reaction with inositol, and catalysed by the enzyme CDP-diacylglycerol inositol phosphatidyltransferase; the other product of the reaction is cytidine monophosphate (CMP). The enzyme is located in the endoplasmic reticulum mainly, although it may also occur in the plasma membrane in yeasts, and almost entirely on the cytosolic side of the bilayer. Phosphatidylinositol is then delivered to other membranes either by vesicular transport or via the agency of specific transfer proteins.



The mechanism for biosynthesis of phosphatidylinositol and phosphatidylglycerol is sometimes termed a branch point in phospholipid synthesis, as phosphatidylcholine and phosphatidylethanolamine are produced by a somewhat different route.

In animal tissues, phosphatidylinositol is the primary source of the arachidonic acid required for biosynthesis of eicosanoids, including prostaglandins, via the action of the enzyme phospholipase A₂, which releases the fatty acids from position *sn*-2.



The reverse reaction also occurs. Lysophosphatidylinositol is formed as a by-product of eicosanoid formation or as an intermediate as part of the normal cycle of deacylation-acylation of phosphatidylinositol in tissues in which the fatty acid composition is remodelled. A membrane-bound *O*-acyltransferase (MBOAT7 or LPAIT1) specific for lysophosphatidylinositol with a marked preference for arachidonoyl-CoA has been characterized from neutrophils. This may be one means by which free arachidonic acid and eicosanoid levels are regulated.

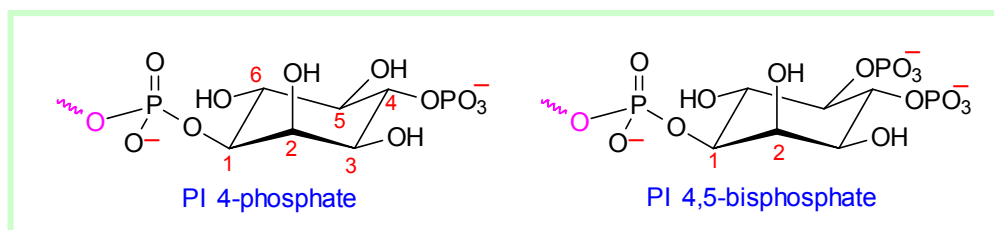
In addition to functioning as negatively charged building blocks of membranes, the inositol phospholipids (including the phosphatidylinositol phosphates or 'polyphosphoinositides' discussed below) appear to have crucial roles in interfacial binding of proteins and in the regulation of proteins at the cell interface. As phosphoinositides are polyanionic, they can be very effective in non-specific electrostatic interactions with proteins. However, they are especially effective in specific binding to so-called 'PH' domains of cellular proteins.

More importantly, phosphatidylinositol and the phosphatidylinositol phosphates (see below) are the main source of diacylglycerols that serve as signalling molecules in animal and plant cells, via the action of a family of highly specific enzymes collectively known as phospholipase C (see our web pages on **diacylglycerols**). They regulate the activity of a group of at least a dozen related enzymes known as protein kinase C, which in turn control many key cellular functions, including differentiation, proliferation, metabolism and apoptosis. Indeed, the biological actions of the various components released have been the subject of intensive study over the last twenty years. **2-Arachidonoyl-glycerol**, an endogenous cannabinoid receptor ligand, may also be a product of phosphatidylinositol catabolism.

Few bacteria appear to contain phosphatidylinositol, although inositol-containing lipids are found in the actinobacteria (lipophosphoglycans – see below). However, **archaeal ether lipids** contain analogues of phosphatidylinositol. In contrast, this lipid is found in all eukaryotes, which are able to synthesise inositol *de novo* via glucose-6-phosphate.

2. Phosphatidylinositol Phosphates

The pioneering work of Mable and Lowell Hokin in the 1950s led to the discovery that phosphatidylinositol was converted to polyphosphoinositides with important signalling and other functional activities in animal cells. This lipid is now known to be phosphorylated by a number of different kinases that place the phosphate moiety on positions 3, 4, and 5 of the inositol ring. Seven different isomers are known, all of which have distinct biological activities. The most significant in quantitative and possibly biological terms were long thought to be **phosphatidylinositol 4-phosphate** and **phosphatidylinositol 4,5-bisphosphate**, but it is now recognized that 3-phosphorylated forms are also extremely important.



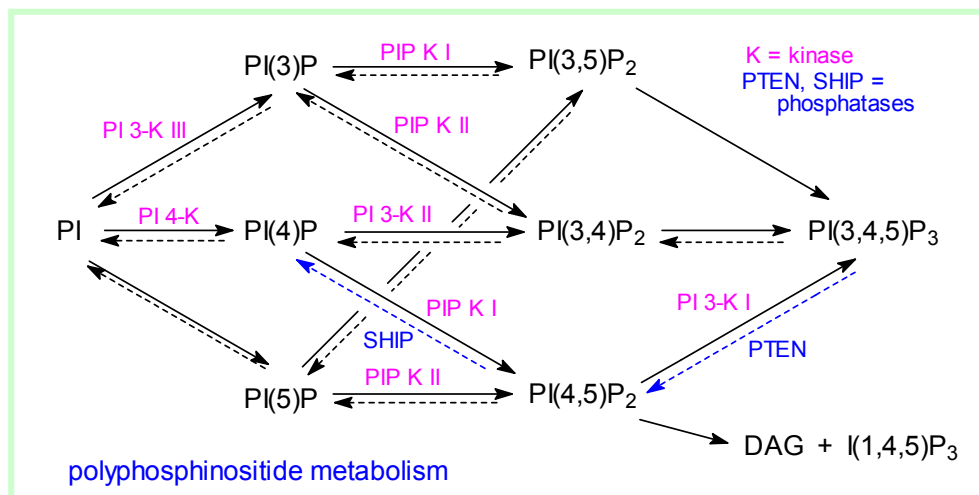
These lipids are usually present at low levels only in tissues, typically at about 0.5 to 1% of the total lipids of the inner leaflet of the plasma membrane, so they are unlikely to have a structural role. The positional distributions of fatty acids in the phosphatidylinositol, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate of ox brain are listed in **Table 2**. In each the saturated fatty acids are concentrated in position *sn*-1 and polyunsaturated, especially arachidonate, in position *sn*-2. There are few differences among the three lipids.

Table 2. Distribution of fatty acids (mol % of the total) in positions *sn*-1 and *sn*-2 in phosphatidylinositol (PI) and the phosphatidylinositol mono- and diphosphates of ox brain.

Fatty acids	PI		PI monophosphate		PI diphosphate	
	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -1	<i>sn</i> -2
16:0	15		9		7	
18:0	74		69		69	
18:1	10	10	20	13	21	10
18:2	1	2	trace	1	1	1
20:3(n-9)		5		10		10
20:3(n-6)		5		11		12
20:4(n-6)		67		49		52
22:3		7		10		7
22:6(n-3)		trace		trace		trace

Holub, B.J., Kuksis, A. and Thompson, W. *J. Lipid Res.*, **11**, 558-564 (1970).

Phosphatidylinositol *per se* is of course the ultimate precursor of all phosphoinositides. The latter are maintained at steady state levels in the inner leaflet of the plasma membrane by a continuous and sequential series of phosphorylation and dephosphorylation reactions by specific kinases, phosphatases and phospholipase C enzymes, which are regulated and/or relocated through cell surface receptors for extracellular ligands. This has been termed a 'futile cycle', and can consume a significant proportion of cellular ATP production. Controlled synthesis of these different phosphoinositides can occur in different intracellular compartments for distinct and independently regulated functions with differing target enzymes. In mammals, the complexity is such that 18 phosphoinositide interconversion reactions have been identified to date, and these are mediated by 19 phosphoinositide kinases and 28 phosphoinositide phosphatases.



Thus, phosphatidylinositol 4-phosphate is produced by the action of a phosphatidylinositol 4-kinase (PI 4-K), and is in turn phosphorylated by a phosphatidylinositol phosphate 5-kinase (PIP K I) to form phosphatidylinositol 4,5-bisphosphate. This can also be formed by phosphorylation of phosphatidylinositol 5-phosphate by a specific 4-kinase (PIP K II). The best characterized of the phosphoinositide signalling functions results from the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C isoforms to produce **diacylglycerols** and inositol-3,4,5-trisphosphate (see below), which act as second messengers. The enzyme activity is stimulated by signalling molecules such as G-protein coupled receptors, receptor tyrosine kinases, Ras-like

GTPases and calcium ions, thus linking the hydrolysis of phosphatidylinositol 4,5-bisphosphate to a wide range of other cellular signals.

Phosphatidylinositol is also phosphorylated by a 3-kinase (PI 3K III) to produce phosphatidylinositol 3-phosphate (three phosphatidylinositol 3-kinases families have been described, each with distinct substrate specificities). It amounts to 0.1-0.5% of the total phosphoinositides in resting mammalian cells. A second phosphoinositide signalling pathway involves activation of two of these 3-kinases, stimulated by growth factors and hormones, which phosphorylate phosphatidylinositol 4,5-bisphosphate (by PI 3-K I) and phosphatidylinositol 4-phosphate (by PI 3-K II) to produce phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate, respectively. In turn, these lipids stimulate signalling pathways involved in cell growth, survival, proliferation, and motility. In particular, they mediate insulin-independent glucose transport and many of the physiological actions of insulin.

The amounts of these various metabolites are also regulated by the activities of specific phosphoinositide phosphatases. For example, so-called 'SHIP' phosphatases convert phosphatidylinositol 4,5-bisphosphate back to phosphatidylinositol 4-phosphate by hydrolysis of the 5-phosphate group.

The various organelles in cells have membranes with distinct functions and molecular compositions. Yet, they are all formed primarily at the endoplasmic reticulum, and the different membrane lipids and proteins must be transported to each site via specific membrane trafficking processes. The concept has emerged in which each phosphoinositide has a specific role – the 'lipid code' hypothesis, in which distinct lipids act as labels for each cellular membrane to maintain the orderly flow required for the complexities of membrane trafficking and spatio-temporal signalling reactions. Thus, phosphatidylinositol 4-phosphate, phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 3-phosphate and phosphatidylinositol 3,5-bisphosphate are found mainly on the Golgi, plasma membrane, early endosomes and late endocytic organelles, respectively, where they are sometimes regarded as landmarks for these compartments. In these various organelles, further phosphorylation via kinases or removal of phosphates via hydrolysis continues.

Phosphoinositides have a central and general position in the fields of cell signalling and regulation. They are able to achieve signalling effects directly by binding to cytosolic proteins or specific cytosolic domains of membrane proteins via their polar head groups. In this way, they can regulate the function of innumerable proteins integral to membranes, for example by relocating a protein from one area of the cell to another, usually from the cytosol to the inner leaflet of the plasma membrane, or they can attract cytoskeletal and signalling components to the membrane. Amongst the proteins that bind to phosphoinositides in this way are phospholipases, protein kinases, regulators of membrane trafficking, and cytoskeletal, scaffold and ion channel proteins.

Binding usually involves electrostatic interactions with the negative charges of the phosphate groups on the inositol ring with characteristic clusters of basic amino acid residues in proteins, and it can lead to folding and thence increased activity of unstructured peptides. In particular, a specific binding region termed the pleckstrin homology (PH) domain, consisting of ~100 amino acids, is the most abundant lipid-binding domain with more than 225 examples identified and exhibits great specificity for several of the polyphosphoinositides. This is driven by non-specific electrostatic interactions initially, but it is followed by specific binding to increase the membrane residence time. The distinctive phosphoinositide composition of membranes in different organelles adds strength and specificity to the interactions by cooperative binding with other membrane proteins.

The **phosphatidylinositol monophosphates** are present in cells at low levels only, although their levels do not appear to fluctuate greatly. Phosphatidylinositol 3-phosphate has been implicated in membrane trafficking through its interactions with specific proteins in endosomes. Indeed, it is a major determinant of the identity of the membrane of early endosomes, and it participates in most

aspects of endosomal function. It may also have a role as a second messenger with an involvement in a number of physiological and pathological processes. Phosphatidylinositol 4-phosphate is the precursor for the 4,5-bisphosphate, but it binds to a protein on the cytoskeleton of the cell and has its own characteristic functions. In particular, it is essential for the structure and function of the Golgi complex, where it is required for the recruitment of specific proteins. Some of these participate in vesicle formation, while others like the oxysterol binding protein are involved in lipid transfer. While the biological properties of phosphatidylinositol 5-phosphate have taken longer to unravel, because of the difficulties of separation of this isomer, it is now apparent that it is involved in osmoregulation both in plants and animals. It may also have signalling functions.

Phosphatidylinositol 4,5-bisphosphate is especially important as a precursor of further metabolites (see below) and because of signalling functions in the plasma membrane in its own right, where it complexes with and regulates many cytoplasmic and membrane proteins, especially those concerned with ion channels for potassium, calcium, sodium and other ions. In most instances, it increases channel activity, while its hydrolysis by phospholipase C reduces such activity. In particular, it appears to interact with cationic residues of specific proteins in concert with cholesterol to form localized membrane domains that are distinct from the sphingolipid-enriched rafts. Also, phosphatidylinositol 4,5-bisphosphate and its diacylglycerol metabolites are important for vesicle formation in membranes. For example, a major pathway for internalization of receptors, such as the transferrin receptor, in cells is the clathrin-coated vesicle pathway. Phosphatidylinositol 4,5-bisphosphate is essential to this process in that it binds to the machinery involved in the membrane, increasing the number of clathrin-coated pits and permitting internalization of transferrin.

Phosphatidylinositol 4,5-bisphosphate is intimately involved in the development of the actin cytoskeleton and its attachment to the plasma membrane, thereby controlling cell shape, motility, and many other processes. In the cell nucleus, this lipid is believed to be involved in maintaining chromatin, the complex combination of DNA, RNA, and protein that makes up chromosomes, in a transcriptionally active conformation, as well as being a precursor for further signalling molecules. It is also an essential cofactor for phospholipase D and so affects the cellular production of **phosphatidic acid** with its specific signalling functions. By binding specifically to ceramide kinase, the enzyme responsible for the synthesis of **ceramide-1-phosphate**, it has an influence on sphingolipid metabolism. Like ceramide-1-phosphate, it binds to and activates the Ca^{2+} -dependent phospholipase A_2 , which generates the arachidonate for eicosanoid production.

The major functions of **phosphatidylinositol 3,5-bisphosphate** are in membrane and protein trafficking, especially in the endosomes. While it is known to be essential to this aspect of cellular metabolism, its precise role is not yet clear. There is recent evidence that it has a role in the responses of mammalian cells to insulin. **Phosphatidylinositol 3,4-bisphosphate** has a role in cell growth and survival; it is known to bind to a specific protein kinase.

Phosphatidylinositol 3,4,5-trisphosphate has been implicated in a variety of cellular functions, such as growth, cell survival, and differentiation. In particular, it is an important component of a signalling pathway in the cell nucleus.

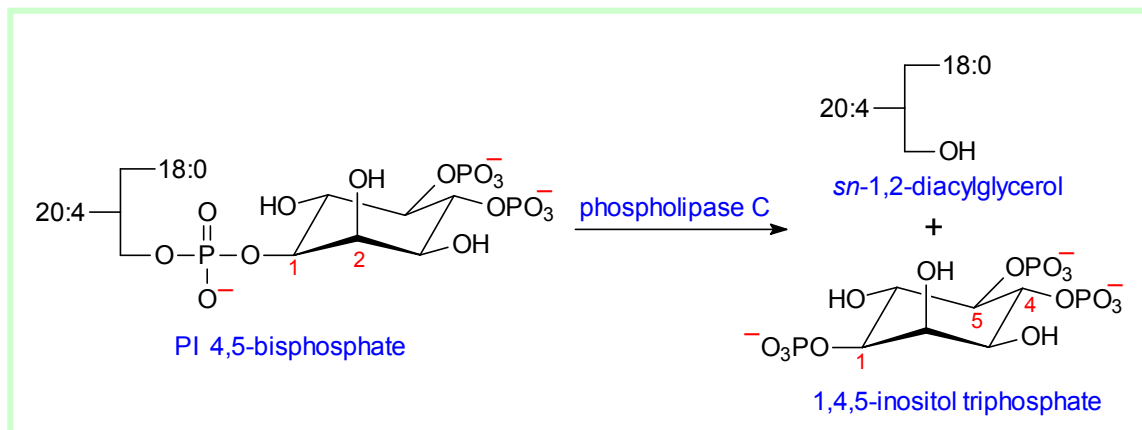
The human immune system utilizes neutrophils, which are highly mobile cells, to eliminate pathogens from infected tissue. The first step is to track and then pursue molecular signals, such as cytokines, emitted by pathogens. It has now been established that two phospholipids operate in sequence to point the neutrophils in the correct direction. The first of these is phosphatidylinositol 3,4,5-trisphosphate, which binds to a specific protein DOCK2 and enables it to translocate to the plasma membrane. Then phosphatidic acid, generated by the action of phospholipase D on phosphatidylcholine, takes over and directs the DOCK2 to the leading edge of the plasma membrane. This causes polymerization of actin within the cell and in effect reshapes the neutrophil and points it in the direction from which the pathogens signals are coming.

In plants as in animals, polyphosphoinositides exert their regulatory effects by acting as specific ligands to regulate enzyme activity. **Phosphatidylinositol 3,4,5-trisphosphate** occurs at low levels in most eukaryotes, but it is the only polyphosphoinositide found in plants under normal growth condition (small amounts of phosphatidylinositol bisphosphate have been detected under conditions of salt stress). Amongst reactions specific to plants are effects on stress adaptation, as well as growth of pollen tubes and root hairs or on guard cell function.

3. Water-Soluble Inositol Phosphates

As mentioned briefly above, hydrolysis of phosphatidylinositol phosphates by five families of enzymes of the calcium-dependent phospholipase C type leads to generation of **sn-1,2-diacylglycerols** (see the appropriate web pages), which act as second messengers in the cell. The other products of this reaction are **water-soluble inositol phosphates**. Up to sixty different compounds are possible, and at least 37 of these have been found in nature at the last count, all of which are also extremely important biologically. However, polyphosphoinositides with a phosphate in position 3 are not substrates for phospholipase C.

For example under the action of various physiological stimuli in animals, inositol 1,4,5-trisphosphate is released from phosphatidylinositol 4,5-bisphosphate, and this is an important cellular messenger, which diffuses into the cytosol and stimulates calcium release from an internal store via ligand-gated calcium channels (the diacylglycerols remain in the membrane to recruit and activate members of the protein kinase C family). The increase in calcium concentration, together with the altered phosphorylation status, activates or de-activates many different protein targets, enabling cells to respond in an appropriate manner to the extracellular stimulus.



All of the various inositol phosphates appear to be involved in the control of cellular events in very specific ways, but especially in the organization of key signalling pathways, the rearrangement of the actin cytoskeleton or intracellular vesicle trafficking. They have also been implicated in gene transcription, RNA editing, nuclear export and protein phosphorylation. As these remarkable compounds can be rapidly synthesised and degraded in discrete membrane domains or even sub-nuclear structures, they are considered to be ideal regulators of dynamic cellular mechanisms. From structural studies of inositol polyphosphate-binding proteins, it is believed that the inositides may act in part at least by modifying protein function by acting as structural cofactors, ensuring that proteins adopt their optimum conformations.

Phosphoinositides and the inositol polyphosphates are key components of the nucleus of the cell, where they have many essential functions, including DNA repair, transcription regulation and RNA dynamics. It is believed that they may be activity switches for the nuclear complexes responsible for such processes, with the phosphorylation state of the inositol ring being of primary importance. Different isomers appear to have specific functions at each level of gene expression, so

extracellular events must coordinate the production of these compounds in a highly synchronous manner.

The extraordinary range of activities of phosphoinositides is relevant to major human diseases, including cancer and diabetes, making them important targets for pharmacological research and intervention.

Less is known of the metabolism of inositol phosphates in higher plants, which lack a receptor for inositol 1,4,5-trisphosphate and in which levels of phosphatidylinositol 4,5-bisphosphate are always extremely low if it is present at all. It is now apparent that inositol hexakisphosphate (phytate) is the cellular messenger that mobilizes an endomembrane store of calcium ions in plants.

4. Glycosyl-Phosphatidylinositol Anchors for Proteins, Lipophosphoglycans and Phosphatidylinositol Mannosides

Phosphatidylinositol is known to be the anchor that links a variety of proteins to the external leaflet of the plasma membrane via a glycosyl bridge (**glycosyl-phosphatidylinositol(GPI)-anchored proteins**). However, this is now considered a sufficiently important topic for its own web page. Lipophosphoglycans (lipoarabinomannans and arabinogalactans) and phosphatidylinositol mannosides are important components of the membranes of parasitic protozoa and bacteria, but for convenience they are also discussed with the GPI-anchored proteins.

5. Lyso-Phosphoinositides

It has long been known that the water-soluble glycerolphosphoinositides, the fully deacylated forms of phosphatidylinositol and the phosphatidylinositol phosphates have key roles in cellular signalling pathways. However, it has become apparent relatively recently that like other lysophospholipids, lysophosphatidylinositol, *i.e.* with a single fatty acid only linked to the glycerol moiety, and the polyphospho-analogues may have messenger functions. They are formed as intermediates in the remodelling of the fatty acid compositions of the lipids, and when arachidonic acid is released for eicosanoid biosynthesis (see above).

6. Analysis

The book by Kuksis cited below is a definitive guide to the topic. Like all acidic phospholipids, phosphatidylinositol is not particularly easy to isolate in a pure state, special care being necessary to ensure that it is fully resolved from phosphatidylserine. However, this can be accomplished by adsorption TLC or HPLC with care. The phosphatidylinositol phosphates are a different matter, however, because of their high polarity and low abundance in tissues. It is necessary to use acidified solvents to extract them efficiently from tissues and to ensure that they are in a single salt form. For isolation of individual components, TLC methods are usually favoured, although detection can be a problem – one approach being to equilibrate with radioactive phosphorus to facilitate detection and quantification by liquid scintillation counting. HPLC with mass spectrometric (electrospray) detection is showing great promise.

Recommended Reading

- o Barlow, C.A., Laishram, R.S. and Anderson, R.A. [Nuclear phosphoinositides: a signaling enigma wrapped in a compartmental conundrum](#). *Trends Cell Biol.*, **20**, 25-35 (2010).
- o Christie, W.W. and Han, X. *Lipid Analysis (4th edition)*. (Oily Press, Bridgwater) (2010).

- o Corda, D. Iurisci, C. and Berrie, C.P. [Biological activities and metabolism of the lysophosphoinositides and glycerophosphoinositols](#). *Biochim. Biophys. Acta*, **1582**, 52-69 (2002).
- o Di Paolo, G. and De Camilli, P. [Phosphoinositides in cell regulation and membrane dynamics](#). *Nature*, **443**, 651-657 (2006).
- o Falasca, M. and Maffucci, T. [Rethinking phosphatidylinositol 3-monophosphate](#). *Biochim. Biophys. Acta*, **1793**, 1795-1803 (2009).
- o Gardocki, M.E., Jani, N. and Lopes, J.M. [Phosphatidylinositol biosynthesis: biochemistry and regulation](#). *Biochim. Biophys. Acta*, **1735**, 89-100 (2005).
- o Kuksis, A. [Inositol Phospholipid Metabolism and Phosphatidyl Inositol Kinases](#). *Laboratory Techniques in Biochemistry and Molecular Biology, Volume 30*, 970 pp. (Elsevier, Amsterdam) (2003).
- o Meijer, H.J.G. and Munnik, T. [Phospholipid-based signalling in plants](#). *Annu. Rev. Plant Biol.*, **54**, 265-306 (2003).
- o Michell, R.H. [Inositol derivatives: evolution and functions](#). *Nature Rev. Mol. Cell Biol.*, **9**, 151-161 (2008).
- o Payrastre, B. [Phosphoinositides](#). In: *Bioactive Lipids*. pp. 63-84. (edited by A. Nicolaou and G. Kokotos, The Oily Press, Bridgwater) (2004).
- o Sasaki, T., Takasuga, S., Sasaki, J., Kofuji, S., Eguchi, S., Yamazaki, M. and Suzuki, A. [Mammalian phosphoinositide kinases and phosphatases](#). *Prog. Lipid Res.*, **48**, 307-343 (2009).
- o Skwarek, L.C. and Boulianne, G.L. [Great expectations for PIP: phosphoinositides as regulators of signaling during development and disease](#). *Developmental Cell*, **16**, 12-20 (2009).
- o Vance, D.E. and Vance, J. (editors) *Biochemistry of Lipids, Lipoproteins and Membranes (4th Edition)*. (Elsevier Science, Amsterdam) (2002) – several chapters.
- o Vicinanza, M., D'Angelo, G., Di Campli, A. and De Matteis, M.A. [Function and dysfunction of the PI system in membrane trafficking](#). *EMBO J.*, **27**, 2457-2470 (2008).

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