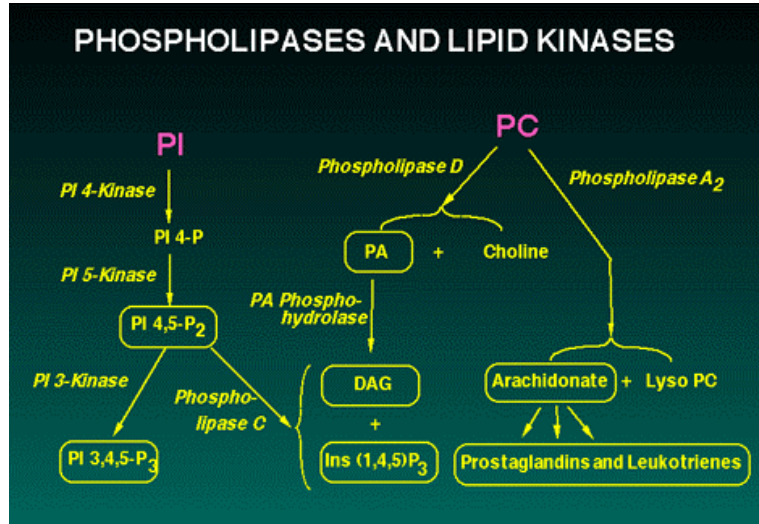


Signal Transduction V Phospholipases

Phospholipases and Signal Transduction. A variety of phospholipid-derived molecules have been implicated as signalling molecules, see rounded boxes in the Figure. These include the protein kinase C regulator diacylglycerol, the calcium signal inositol 1,4,5-trisphosphate, probably phosphatidic acid, and phosphorylated forms of phosphatidylinositol. The phospholipase C system generates diacylglycerol and IP₃ from phosphatidylinositol 4,5-bisphosphate. Phospholipase D catalyzes the hydrolysis of phosphatidylcholine to form phosphatidic acid and released choline headgroup. The phosphatidic acid may itself act as a signal molecule (e.g., by activating a PA-activated kinase), or can be hydrolyzed to form diacylglycerol by the enzyme PA phosphohydrolase. (Figure taken from JD Lambeth, Emory University)



Phospholipases and Phospholipids in Signal Transduction

Phospholipases and phospholipids are involved in the processes of transmitting ligand-receptor induced signals from the plasma membrane to intracellular proteins. The primary protein affected by the activation of phospholipases is PKC which is maximally active in the presence of calcium ion and DAG. The generation of DAG occurs in response to agonist activation of various phospholipases. The principal mediators of PKC activity are receptors coupled to activation of phospholipase C-g (PLC- β). PLC- β contains SH2 domains that allow it to interact with tyrosine phosphorylated Receptor Tyrosine Kinases (RTKs). This allows PLC- β to be intimately associated with the signal transduction complexes of the membrane as well as membrane phospholipids that are its substrates. Activation of PLC-g leads primarily to the hydrolysis of membrane phosphatidylinositol bisphosphate (PIP₂) leading to an increase in intracellular DAG and inositol trisphosphate (IP₃). The released IP₃ interacts with intracellular membrane receptors leading to an increased release of stored calcium ions. Together, the increased DAG and intracellular free calcium ion concentrations lead to increased activity of PKC.

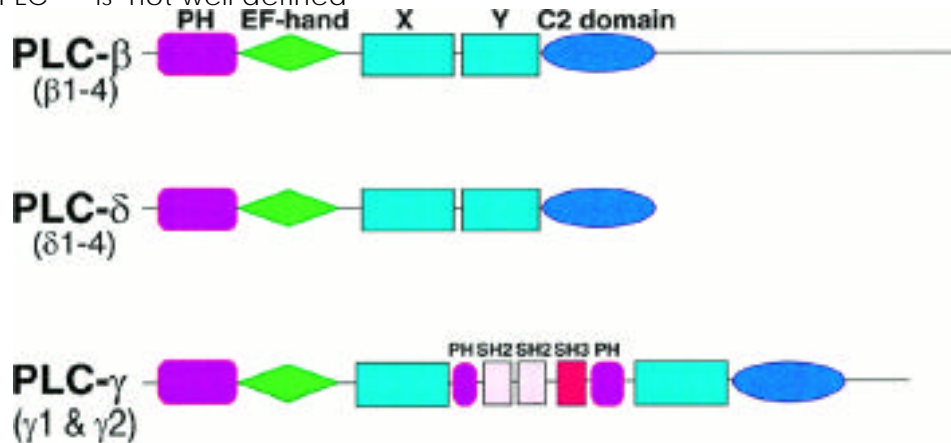
Recent evidence indicates that phospholipases D and A₂ (PLD and PLA₂) also are involved in the sustained activation of PKC through their hydrolysis of membrane phosphatidylcholine (PC). PLD action on PC leads to the release of phosphatidic acid which in turn is converted to DAG by a specific phosphatidic acid phosphomonoesterase. PLA₂ hydrolyzes PC to yield free fatty acids and lysoPC both of which have been shown to potentiate the DAG mediated activation of PKC. Of medical significance is the ability of phorbol ester tumor promoters to activate PKC directly.

Phospholipase C

The lipase activity regulates PIP₂ signaling and is used by many different hormones.-So how is PLC regulated?

Which signaling systems depends on PLC isozyme

- 8 different in 4 families PLC proteins / genes
- PLC β - activated by growth factors & insulin -> SH2 SH3 domains
- PLC δ enzymes activated by Gq proteins some by β subunits
- PLC γ - is not well defined



Differences in structural organization of forms of phospholipase C

- X and Y domains conserved throughout the PLC family - make up the catalytic domains
- Each isozyme contains a PH (plekstrin homology domain) for binding inositol lipids
- Calcium binding occurs through EF hand domain for each form and a C2 domain also interacts with calcium
- PLC γ - has additional SH domains (SH2 and SH3) for interactions with phosphotyrosine signaling

Calcium is required for activity, binding both at C2 domain and at the active site via histidine residues. Inositol lipids bind to protein, Ca²⁺ lead to X and Y domain coming together to form active catalytic protein

Activation of PLC beta form

- Activated by hormones which signal through Gq
- Hormones include angiotensin II, alpha adrenergic agonists, acetylcholine
- Lipase acts as GAP for G protein alpha subunit
- G proteins interact at the C terminal
- beta gamma subunits of G proteins also can activate PLC beta

Activation of PLC gamma

- receptors which bind growth factors lead to PLC gamma activation
- Interactions between intracellular domain of Receptor and PLC occurs by the SH2 domains
- Once interacting PLC gamma becomes phosphorylated by the receptor on tyrosine residues - increases PLC interactions with other proteins
- SH3 domain targets activated PLC to cytoskeletal proteins (actin myosin)
- In immune cells soluble tyrosine kinase can activate by phosphorylation of PLC

PLC inactivation: Additional phosphorylation of PLC leads to inhibition and down regulation of PLC signal - by either PKA or PKC

Phospholipase A2

The phospholipase A2 (PLA2) enzymes hydrolyze fatty acid from the sn-2 position of phospholipid with the concomitant production of lysophospholipid.

Mammalian cells contain structurally diverse forms of PLA2 including secretory PLA2 (sPLA2), calcium-independent PLA2, and the 85-kDa cytosolic PLA2 (cPLA2) (1-4).

PLA2 functions in the digestion of dietary lipid, microbial degradation, and regulation of phospholipid acyl turnover either in a housekeeping role for membrane repair or for the production of inflammatory lipid mediators.

Phospholipase activity was first described in pancreatic juice and cobra venom at about the turn of the century.