

Phospholipase D

Key References

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Overview

Phosphatidylcholine (PC)-specific phospholipase D (PLD) enzymes catalyze the hydrolysis of PC to generate choline and phosphatidic acid. These enzymes belong to a superfamily of phosphatidyltransferases with a characteristic unique duplicated catalytic domain motif, the PLD or "HKD" domain flanked by two conserved regions that are also critical for catalysis and postulated to be important for substrate recognition. PLD isoenzymes have been identified in species from bacteria to mammals. Some bacterial PLDs have broader substrate specificity than the eukaryotic enzymes and may play roles in pathogenesis. Three plant PLD isoenzymes (PLD- α , PLD- β and PLD- γ) have been identified by cDNA cloning. These differ in their dependence on calcium and phosphoinositides for activity and have been suggested to be important in membrane remodeling during development or wound repair. In budding yeast, PLD activity encoded by the *spo14* gene is required for formation of the prospore membrane during meiosis and has a conditional role in regulation of secretion during vegetative growth.

Mammalian PLD enzymes are effector enzymes in signal transduction pathways initiated by heterotrimeric G protein-coupled cell surface receptors and receptor tyrosine kinases. A number of intracellular and extracellular messenger functions for phosphatidic acid have been described in mammalian cells. These include regulation of protein and lipid kinases, cell adhesion receptors; the phagocyte burst oxidase, the target of rapamycin (TOR) and guanine nucleotide exchange proteins for small GTPases. For example, phosphatidic acid activates phosphatidylinositol 4-phosphate 5-kinase, which may underlie localized synthesis of PI (4,5)P₂ linked to mem-

brane ruffling in the plasma membrane of growth factor stimulated cells or effects on membrane transport and exocytosis, while activation of the Raf protein kinase and TOR by this lipid may link PLD activation to control of cell proliferation and survival. Phosphatidic acid can also be metabolized by dephosphorylation to produce diacylglycerol for activation of PKC isoenzymes or deacylated by phospholipase A₂ to generate lyso-phosphatidic acid which is active at cell surface receptors. The downstream pathways regulated by PLD1 and PLD2 suggest roles in disease states including cancer, inflammation, cardiovascular disease and diabetes. However although some evidence for aberrant disease-specific expression and regulation of PLD activity has been presented, a defined role for PLD1 or PLD2 in these disease states has not yet been established.

With the possible exception of the unidentified enzyme responsible for a fatty acid stimulated PLD activity, two mammalian PLD gene products, PLD1 and PLD2 account for the PLD activities described in mammalian cells and tissues. PLD1 and PLD2 are ubiquitously expressed although relative levels of the two enzymes vary considerably between cell types. PLD1 and PLD2 display cell type specific and dynamically regulated differences in localization between the plasma membrane and intracellular membranes. Control of PLD1 activity by protein and lipid modulators is complex. Both *in vitro* and intact cell studies suggest roles for GTP-binding proteins of the ADP-ribosylation (ARF) and Rho families and PKC in this process. PLD2 is insensitive to these activators and activity of this enzyme may be controlled by mechanisms involving protein inhibitors that have been identified using *in vitro* assays. These include fodrin,

synaptojanin, clathrin assembly protein-3 and synucleins.

PLD enzymes operate by an ordered bisubstrate or "ping-pong" mechanism with a covalent enzyme-phosphatidic acid intermediate. Primary alcohols can substitute for water in the product release step of the reaction cycle and, in this case, the enzyme catalyzes a transphosphatidyl transfer reaction forming a metabolically stable phosphatidyl-alcohol. This reaction is highly efficient. In the absence of specific inhibitors of PLD enzyme activity, primary alcohols have therefore been used to antagonize PLD signaling by inhibiting phosphatidic acid production and promoting formation of phosphatidylalcohols, which are presumed to be inert in intact cells. A widely used inhibitor of conventional PKC enzymes, calphostin-c, is a potent direct inhibitor of PLD1 and PLD2 with less dramatic effects on the activity of plant and bacterial PLDs

Phospholipase D

CLASS	← Bacterial →		← Yeast →	
		Streptomyces (P8023)	<i>Corynebacterium</i>	spo14p
MOLECULAR WEIGHT (kDa)	59.0	33.6	195.2	ND
SUBSTRATE SPECIFICITY	PC (P3841, P6638), SM (S7004), Lyso-PC (L1381)	Not known	PC (P3841, P6638)	PS (P5660, P6641, P7769) > PE (P9137, P7693) > PC (P3841, P6638)
METAL ION DEPENDENCE	Ca ²⁺ Mg ²⁺	Ca ²⁺ Mg ²⁺	Inhibited by Mg ²⁺	Not known
ACTIVATORS/COFACTORS	Not known	Not known	PI(4,5)P ₂ (P9763)	Not known
TISSUE EXPRESSION	NR	NR	Increased in meiosis	Not known
PHYSIOLOGICAL FUNCTION	Pathogenesis?	Pathogenesis?	Meiosis, secretion	Not known
DISEASE RELEVANCE	NR	NR	NR	NR

Phospholipase D (continued)

CLASS	← Plant →			← Mammalian →		
		PLD-α	PLD-β	PLD-γ	PLD1	PLD2
MOLECULAR WEIGHT (kDa)	92.0	108.6	95.5	124.2 (1a) 118.7 (1b)	106.1	ND
SUBSTRATE SPECIFICITY	PC (P3841, P6638)	PC (P3841, P6638)	PC (P3841, P6638)	PC (P3841, P6638)	PC (P3841, P6638)	PC (P3841, P6638)
METAL ION DEPENDENCE	Ca ²⁺ (mM) Mg ²⁺	Ca ²⁺ (μM) Mg ²⁺	Ca ²⁺ (μM) Mg ²⁺	Ca ²⁺ Mg ²⁺	Ca ²⁺ Mg ²⁺	Ca ²⁺ Mg ²⁺
ACTIVATORS/COFACTORS	Not known	Not known	PI(4,5)P ₂ (P9763) ARF Rho PKC (P7956, P0329)	PI(4,5)P ₂ (P9763)	Free fatty acids	Not known
PROTEIN INHIBITORS	Not known	Not known	Fodrin Synaptojanin AP3	Synuclein Synapotojanin	Not known	Not known
SMALL MOLECULE INHIBITORS (NOT SPECIFIC)	Not known	Not known	Ceramides (C2137), primary alcohols, Calphostin-c	Not known	Alkylphosphocholines (H6772),	Not known
TISSUE EXPRESSION	Vegetative tissues	Vegetative tissues	Vegetative tissues	Ubiquitous	Ubiquitous	Not known
PHYSIOLOGICAL FUNCTION	Stress responses?	Stress responses?	Stress Responses?	Signaling/ membrane trafficking		Not known
DISEASE RELEVANCE	Wound healing/ response to pathogens/ freezing tolerance?			Inflammation, cardiovascular disease cancer, diabetes?		Not known

Abbreviations

AP3: Clathrin assembly protein-3
ARF: ADP-ribosylation factor family GTP-binding proteins
Lyso-PC: Lyso-Phosphatidylcholine
ND: Not determined

PE: Phosphatidylethanolamine
PLD: Phosphatidylcholine-specific phospholipase D
spo14p: *S. cerevisiae* spo14 gene product
PC: Phosphatidylcholine
PI(4,5)P₂: Phosphatidylinositol 4,5-bisphosphate

PS: Phosphatidylserine
Rho: Rho family GTP-binding proteins
SM: Sphingomyelin
NR: Not relevant