Minireview

The Role of Phosphatases in Inositol Signaling Reactions*

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The phosphatidylinositol signaling pathway employs a host of kinases and phosphatases that form and degrade the many signaling molecules that act in this system. The complexity and robustness of the system are indicated by the fact that there are six inositol phospholipids and more than 20 soluble inositol phosphates that have been found in mammalian cells (1). The system is present in all eukaryotic cells in one form or another. This review will focus on a few of the phosphatases as they exist in mammalian cells. In general, the inositol phosphatases are analogous to protein phosphatases in that they both tend to inhibit or terminate signaling reactions. There are specific exceptions to this general rule in both systems. Numerous inositol phosphatases have been discovered and characterized in the past 15 years. Surprisingly, in many cases the same enzymes hydrolyze phosphate from both water-soluble inositol phosphates and the corresponding lipids with the same arrangement of phosphate groups.

Inositol Polyphosphate 5-Phosphatases

The first 5-phosphatase enzymes studied were those that hydrolyze inositol 1,4,5-trisphosphate (Ins-1,4,5-P_3) to inositol 1,4-bisphosphate (Ins-1,4-P_2) (1–3). Because the former stimulates cellular calcium mobilization whereas the latter does not, this reaction serves to terminate calcium signaling. In initial attempts to purify 5-phosphatase from human platelets, two peaks of activity were eluted from DEAE-cellulose (4, 5). This was the first indication that multiple 5-phosphatase enzymes exist, and the platelet enzymes were designated as types I and II. There are now numerous 5-phosphatase enzymes that have been cloned and characterized (6). All are magnesium-dependent phosphomonoesterases. There are eight distinct mammalian enzymes that are products of different genes for which cDNA clones have been isolated (Fig. 1). In addition, several of these cDNAs exist in alternatively spliced versions, the significance of which is the most part unknown. 5-Phosphatases are also found in plants, Caenorhabditis elegans, and Drosophila, and there are four yeast genes that encode 5-phosphatase enzymes (6, 7). We have classified the 5-phosphatases according to their substrate specificity into four groups. These enzymes are designated as inositol polyphosphate 5-phosphatases as the known substrates have multiple phosphate groups. There are four known substrates for 5-phosphatases: Ins-1,4,5-P_3, Ins-1,3,4,5-P_4, and the lipids PtdIns-4,5-P_2 and PtdIns-3,4,5-P_3. 5-Phosphatases are homologous with varying degrees of sequence conservation (8). They are defined by two signature motifs: (P/I)WGDXXN(F/Y)R and (R/N)XP(S/A)W/Y)C/T)DR (IV/IA) as shown in Fig. 1. These are separated by 60–75 amino acids except for 5-phosphatase I, where the motifs are 103 amino acids apart.

Group I 5-phosphatases hydrolyze only the water-soluble substrates Ins-1,4,5-P_3 and Ins-1,3,4,5-P_4. They are the most active against these substrates of all 5-phosphatases and probably function as the enzymes that terminate calcium signaling. The platelet type I enzyme is a representative of this group, which includes members cloned from dog thyroid and placenta (9, 10). These enzymes are membrane-associated presumably through isoprenylation (11, 12). 5-Phosphatase I in platelets is in a stoichiometric complex with pleckstrin that regulates its activity (13). When platelets are stimulated with thrombin, pleckstrin is rapidly phosphorylated on serine and threonine residues by protein kinase C, and this activates the 5-phosphatase. Another signaling molecule, 14-3-3 protein, is also found in this complex (14). Cells stably transfected with an antisense cDNA for 5-phosphatase I underexpress the enzyme, have elevated levels of Ins-1,4,5-P_3 and elevated basal intracellular calcium levels, and display a transformed phenotype further supporting the hypothesis that group I enzymes control calcium signaling (15).

Group II enzymes hydrolyze all four 5-phosphatase substrates although with varying catalytic efficiency. Platelet type II 5-phosphatase belongs to this group and was the first 5-phosphatase cloned (16). OCRL-1 is the X chromosome-encoded gene that when mutated causes Lowe syndrome or oculocerebrorenal dystrophy (17). Platelet 5-phosphatase II and OCRL-1 are 51% identical over a span of 744 amino acids and OCRL-1 is a 5-phosphatase (18). Lowe syndrome is characterized by renal tubular acidosis, cataracts, and mental retardation; renal failure and retinal degeneration develop inexorably by early adult life (19). Both of these enzymes have about 300 amino acids of nonhomologous sequence N-terminal to the 5-phosphatase catalytic domain. These extra sequences are not required for enzyme activity and presumably provide for localization or association with other proteins. Both of these enzymes are membrane-associated without membrane-spanning domains. 5-Phosphatase II is isoprenylated (11) and is bound to mitochondria and plasma membrane (20). OCRL-1 is not modified by any lipid moiety and is found on the surface of lysosomes (21) although in lymphocytes it was reported to be bound to the Golgi apparatus (22). In preliminary studies using renal proximal tubule cell lines from a patient with Lowe syndrome, lysosomal enzymes appear to be missorted. Extracts of these cells are deficient in PtdIns-4,5-P_2 and PtdIns-3,4,5-P_3 hydrolytic activity, whereas the corresponding water-soluble inositol phosphates are hydrolyzed normally, indicating that OCRL-1 is a lipid phosphatase (21).

There is considerable evidence that PtdIns-4,5-P_2 is required for budding of membrane vesicles from lysosomes, and the...
accumulation of this lipid may afford abnormally increased trafficking of enzymes from lysosomes to the extracellular space (23). Disruption of OCRL-1 in mice leads to no abnormal phenotype, and 5-phosphatase II-deficient mice also appear normal. When these mutants were interbred, deficiency of both enzymes resulted in an embryonic lethal phenotype (24). This result suggests that the enzymes have overlapping functions in mice, although apparently not in humans, because OCRL-1-deficient proximal tubule cell lines from a Lowe syndrome patient express 5-phosphatase II and still have a severe defect in PtdIns-4,5-P2 metabolism. Thus, these cells accumulate Ptd-Ins-4,5-P2, presumably on lysosomes, and lysosomal enzymes accumulate extracellularly. Perhaps the lifelong leakage of enzymes from lysosomes leads to tissue damage and eventually causes renal failure and blindness.

Synaptojanin (25–28) and synaptojanin 2 (29, 30) are related group II enzymes that participate in synaptic vesicle trafficking. These enzymes occur in several alternatively spliced forms of uncertain significance (30). Synaptojanin forms complexes with dynamin and amphiphysin to promote synaptic vesicle recycling. Synaptojanin contains C-terminal proline-rich regions that can bind to SH3 domains. Synaptojanins contain a domain that is homologous to the yeast protein SacI, which suppresses mutations in yeast Sec14 phosphatidylinositol transfer protein (31). The two synaptojanins are over 50% identical in the SacI and 5-phosphatase domains.

Group III enzymes hydrolyze only substrates with a 3-position phosphate group (i.e., Ins-1,3,4,5-P4 and PtdIns-3,4,5-P3). There are two such enzymes designated as SHIP and SHIP2 (32–34). They are 50% identical in amino acid sequence but have very different tissue distributions. They contain the N-terminal SH2 domain and phosphotyrosine binding domains and upon activation of receptors form complexes with cellular signaling proteins. SHIP is expressed only in cells of hematopoietic origin and participates in signaling through essentially all hematopoietic cytokine receptors including the erythropoietin, interleukin-3, and colony-stimulating factor receptors, as well as various mast cell and B and T cell receptors (35). SHIP forms complexes with the cellular signaling molecules Grb2 and Shc and with the ITIM motif on receptor-ligated FcγRII (36–38). The enzyme hydrolyzes the signaling molecule PtdIns3,4,5-P3 that is presumed to stimulate signaling by activating Akt, protein kinase C, and other targets. Thus, SHIP has a negative function and terminates signals. The targeted disruption of SHIP in mice leads to excessive cytokine signaling, myeloid proliferation, and death from infiltration and consolidation of the lungs by myeloid cells (39). SHIP2 is expressed widely in nonhematopoietic cells and serves a similar function inhibiting cellular responses to insulin, epidermal growth factor, and platelet-derived growth factor (40).

Group IV 5-phosphatases remain poorly characterized and are an activity that only hydrolyzes PtdIns-3,4,5-P3 (41) and forms complexes with PtdIns 3-kinase. We have recently isolated a cDNA encoding a Group IV enzyme (as shown in Fig. 1).

Inositol Polyphosphate 1-Phosphatase

In contrast to the large 5-phosphatase gene family, there is a single gene that encodes inositol polyphosphate 1-phosphatase. It is a member of a magnesium-dependent, lithium-inhibited gene family that is characterized by a common structural fold (42). The enzyme hydrolyzes Ins-1,4-P2 and Ins-1,3,4-P3 and does not act on lipid substrates. Inositol monophosphatase is another member of this family, although it does not share significant amino acid identity except in the active site region (43). Lithium is used to treat manic depressive disease. A popular though unsubstantiated theory is that monophosphatase is the target of lithium action and serves to trap inositol monophosphates. This could deplete cells of free inositol leading to inhibition of inositol signaling reactions. The recent targeted deletion of 1-phosphatase in Drosophila suggests that lithium may inhibit this enzyme in vivo (44). The 1-phosphatase-deficient flies have a "shaker" phenotype and
dramatic defects in synaptic transmission. Treatment of wild-type flies with lithium produces phenocopies of the knockout flies, indicating that lithium inhibits 1-phosphatase in vivo. Treatment of 1-phosphatase knockout flies with lithium had no further effect. Responsiveness to lithium in patients with bipolar disorder has been linked to 1-phosphatase (45). Thus 1-phosphatase is a bona fide target for the therapeutic action of lithium, and other inhibitors of this enzyme might be used in the treatment of this disease.

**Inositol Polyphosphate 4-Phosphatase**

The inositol polyphosphate 4-phosphatases are a family of enzymes implicated in the regulation of PtdIns 3-kinase signaling. These magnesium-independent phosphatases catalyze the hydrolysis of the 4-position phosphate of the second messenger, PtdIns-3,4-P_2, yielding the product PtdIns-3-P (46). 4-Phosphatase cDNA clones have been isolated that are derived from two 4-phosphatase genes. 4-Phosphatase type I and II cDNAs encode proteins that are 37% identical and contain the conserved motif CKSADRT (47). This motif contains the active site consensus sequence C-X_3-R identified for other magnesium-independent phosphatases (48, 49). The conserved cysteine is the essential catalytic nucleophile for C-X_3-R phosphatases (50), and site-directed mutation of this cysteine to serine results in inactivation of 4-phosphatase (46). Both 4-phosphatase I and II transcripts are alternatively spliced resulting in the expression of proteins with putative transmembrane domains near their C termini (47). 4-Phosphatase anti- serum that cross-reacts with the hydrophilic C-terminal spliceforms of 4-phosphatase I and II immunoprecipitates >95% of observed PtdIns-3,4-P_2 phosphatase activity from human platelets, rat brain, heart, skeletal muscle, and spleen supernatants, suggesting that these are major enzymes for the metabolism of this second messenger (50). Moreover, stimulation of human platelets with thrombin or calcium ionophore results in inactivation of 4-phosphatase I by proteolytic cleavage mediated by the calcium-dependent protease calpain (51). This inactivation of 4-phosphatase correlates with the calcium/aggregation-dependent accumulation of PtdIns-3,4-P_2 characteristic of stimulated human platelets. This result implies 4-phosphatase is involved in the regulation of this lipid second messenger (51).

A recent study suggests a role for an inositol phosphate phosphatase in the virulence of *Salmonella* infections. SopB, a protein secreted by *Salmonella dublin*, was shown to have sequence similarity to the 4-phosphatase active site motif (52). SopB is transported into the cytoplasm of host cells by the *Salmonella* Inv/Spa type III secretion system and has been shown to contribute to the virulence of *S. dublin* infection by increasing intestinal fluid secretion and neutrophil recruitment (53). SopB encodes a phosphatase that hydrolyzes many but not all inositol phosphates including inositol 1,3,4,5,6-pentakisphosphate yielding the product inositol 1,4,5,6-tetrakisphosphate (Ins-1,4,5,6-P_4). Ins-1,4,5,6-P_4 has been shown to increase chloride secretion by antagonizing PtdIns-3,4,5-P_3 inhibition of the calcium-dependent chloride channel, and infection of HeLa cells with *S. dublin* results in a characteristic increase of Ins-1,4,5,6-P_4 levels (54). Mutation of the SopB active site cysteine to serine abolishes phosphatase activity. Infection of HeLa cells with a *S. dublin* strain that expresses the inactive mutant SopB fails to elevate Ins-1,4,5,6-P_4 levels (53). Infection of calf ileal loops with Cys → Ser S. dublin results in greatly reduced diarrhea or neutrophil accumulation, thus indicating that enzyme activity per se accounts for virulence. These findings suggest that SopB contributes to *Salmonella* virulence by disruption of the inositol metabolism of the host cell. Although the SopB phosphatase is related to 4-phosphatases, it has a much broader substrate specificity.

A human tumor suppressor gene designated as PTEN was recently shown to be an inositol phosphatase (55). It has been proposed that this enzyme suppresses cell proliferation by hydrolyzing PtdIns-3,4,5,6-P_4 (56). In our unpublished study of this enzyme we find that it has rather broad substrate specificity similar to SopB. A metal ion-independent mammalian phosphatase of broad substrate specificity has been cloned recently and designated as MIPP or multiple inositol polyphosphate phosphatase (57). MIPP has no similarity to any other inositol phosphatase.

**Conclusion**

Roles for inositol phosphate phosphatases in diverse cellular signaling reactions have been discovered recently. Additional study of these enzymes will further elucidate the pathways of intracellular signaling.
Minireview: Inositol Phosphate Phosphatases

10672

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