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Minireview

Isozymes delta of phosphoinositide-specific phospholipase C*°

Tadeusz Pawełczyk✉

Department of Molecular Medicine, Medical University of Gdańsk, Gdańsk, Poland

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Phospholipase C (PLC, EC 3.1.4.11) is the major starting point in the phosphatidylinositol pathway, which generates intracellular signals that regulate protein kinase C and intracellular calcium concentration. To date, three major types of phosphoinositide-specific PLC species named β, γ and δ, have been characterized. This article reviews recent studies on isozymes delta of PLC. Four such isozymes have been cloned and termed δ_{1-4}. Their structural organization, regulation of activity and the interaction with membrane lipid are considered. The intracellular localization of delta isozymes and distribution in various tissues are presented. Attention is given to the pathological conditions in which an abnormal protein level of PLC δ or its activity have been observed.

Phosphoinositide-specific phospholipase C (PLC) is the key enzyme involved in signaling at the plasma membrane of most eukaryotic cells. PLC hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield two second messengers, namely inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. The latter mediates activation of protein kinase C, while IP_3 activates the release of Ca^{2+} into the cytosol and thereby regulates Ca^{2+}-dependent processes [1, 2].

Three major types of phosphoinositide-specific PLC called β, γ and δ, have been characterized, of which four PLC β, two PLC γ and four PLC δ isozymes are known [3, 4]. All mammalian PLC isozymes are single polypeptides.

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✉Correspondence should be addressed: Department of Molecular Medicine, Medical University of Gdańsk, ul. Dębinki 7, 80-211 Gdańsk, Poland; tel: (48 58) 349 2759, fax: (48 58) 344 9653, e-mail: tkpaw@amedec.amg.gda.pl

Abbreviations: PH domain, pleckstrin homology domain; PLC, phospholipase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol-1,4,5-trisphosphate.

STRUCTURAL ORGANIZATION OF PLC δ ISOZYMES

All four δ isozymes have been cloned. PLC δ2 and PLC δ4 were expressed in COS-1 and HeLa cells, respectively [5, 6]. However, all the structural work was so far performed with PLC δ1 and PLC δ3. This is because both these isozymes in active form can be expressed in high yield in *E. coli* cells, and then can be readily purified to homogeneity [7–10]. The δ-type isozymes with molecular masses within the range of 83–87 kDa, are the smallest PLC isozymes. Comparison of the amino-acid sequences of four δ isozymes reveals that they are identical in 45% to 84% [10]. Analysis of δ-type isozymes shows that each of these δ isoforms has in its NH_2-terminal region a pleckstrin homologous domain (PH), preceding the EF-hand region (Fig. 1). The pleckstrin homologous domain is used for attachment of the enzyme to cellular membranes by binding to the IP_3 moiety of PIP_2 [11–14]. The purpose of the EF-hand region is not clear. Examination of the X-ray structure of δ1 isozyme revealed that this region contains calcium ligands [15]. However, deletion of the EF-hand domain in PLC δ1 had no effect on the calcium dependence of the enzyme activity [16]. The catalytic center of phosphoinositide-specific PLC's is located in the regions of high sequence homology named X and Y. The three-dimensional structure of PLC δ1 shows that the X and Y domains form a TIM-barrel-like structure [15]. In δ isozymes the two halves of this structure are connected by an unconserved region rich in acidic amino acids (Fig. 2). Experiments with deletion mutants and active fragments of PLC δ1 generated by limited proteolysis showed that modulation of PLC δ1 activity by positively charged molecules (sphingosine, polyamines) depends on interaction of these compounds with the region spanning X and Y domains [16, 17]. The structural work on δ1 isozyme revealed the existence, on its C-terminus, of a putative membrane-binding C2 domain which contains multiple binding sites for calcium and other metal ions [15, 18, 19]. Typically the C2 domains are modules of about 120 residues identified in more than 40 proteins, many of which are involved in signal transduction and membrane interaction [20]. It is postulated that the C2 domain of δ isozymes could be involved in calcium-dependent phospholipid binding. Essen *et al.* [19] proposed that the C2 domain assists in proper positioning of the catalytic domain of PLC toward the substrate located in the membrane. The multidomain organization of the structure of δ isozymes is likely to be common to all mammalian phosphoinositide-specific PLC's, although β

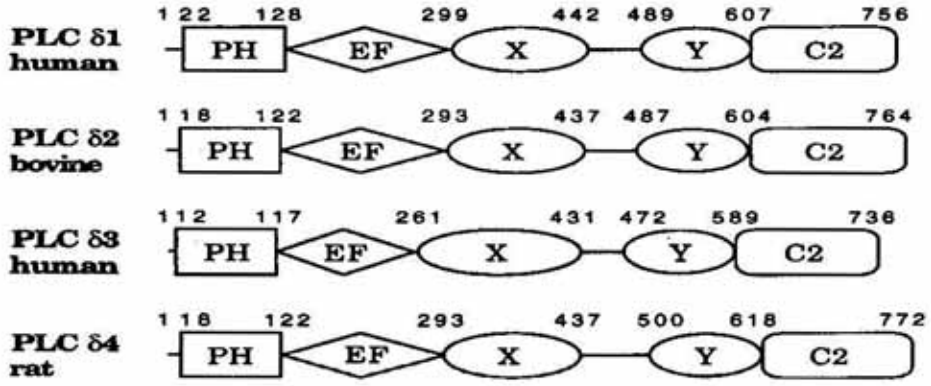

Figure 1. Linear representation of the phospholipase C (PLC) δ isozymes.

X and Y represent regions of high sequence homology, which constitute the PLC catalytic domain; the numbers above refer to the first and last amino acids.

PLC δ1	GGLLPPGGEGGPEATVVSDEDEAAEMEDEAVRSRVQHKPKEDKLRL	(443-488)
PLC δ2	MLEEEEEEPEAELEAEQEARLDLEAQLESEPQDLSPRSEDKKKKPKAIL	(438-486)
PLC δ3	PARSEDGRALSDREEEEEDDEEEEEEVEAAAQRRLAKQI	(432-471)
PLC δ4	RTIEVVESDKEEEELEKDEGSDLDPASAELDMQSQPESQEQASGNKSKNKKKFLLGSSTTIL	(438-499)

Figure 2. Sequences of the region that spans the X and Y domains in PLC δ isozymes.

The sequences were deduced from the cDNA clones reported for δ1 (Kriz *et al.* [22]), δ2 (Meldrun *et al.* [5]), δ3 (Kriz *et al.* [22]) and δ4 (Lee & Rhee [6]). The numbers in parentheses refer to the first and last amino acids. Amino acids with side chains containing acidic groups are shown in bold.

and γ isoforms contain additional regulatory domains [4].

LOCALIZATION IN THE CELL AND TISSUE DISTRIBUTION

PLC δ1 was isolated and purified from several animal sources. It was originally cloned from W138 cells, a line of human fibroblasts [21, 22], from rat brain [23], and subsequently from several other animal tissues [24, 25]. To date PLC δ2 was isolated and purified only from bovine brain [26]. PLC δ3 and PLC δ4 were identified based on their cDNA. PLC δ3 was cloned from W138 cells [21, 22], and PLC δ4 from rat brain and liver [6, 27]. Relatively little is known about localization of PLC δ isozymes in the cell. It has been reported that the δ1 and δ3 isozymes are located in the cytosol and in the membrane fraction of various cells [28-32]. However, the amount of each isozyme in the cellular fractions differs significantly. In human platelets and rat liver δ1 isozyme is present mainly in the cytosolic fraction, and only a minute amount of PLC δ1 can be detected in the membrane fraction. In contrast, the amount of PLC δ3 in cytosolic fraction of the cells is significantly lower than that detected in the membrane fraction [32]. PLC δ1 and PLC δ3 are absent from nuclei of rat liver cells [32]. Recently it has been reported that PLC δ4 is predominantly located in the nucleus [27]. The level of PLC δ4 expression depends strongly on the cell cycle. The nuclear content of PLC δ4 increases upon transition from the G1 to the S phase, and remains high until the end of the M phase. At the beginning of the next G1 phase, PLC δ4 almost disappears [27]. The expression level of PLC δ isozymes was examined in various tissues. In all of them δ1 expression was strongest. The immunoblot analysis revealed that expression of PLC δ3 in rat kidney, cardiac muscle and aorta was higher than its expression in spleen, liver and brain [32]. In the rat, the amount of PLC δ4 was the highest in testis and decreased in the following order: brain > skeletal muscle > thyroid gland > stomach > thymus > aorta > heart [6]. In kidney, liver, prostate, adrenal gland, intestine, pancreas, and lung the PLC δ4 protein is undetectable. Very little is known on the tissue distribution of PLC δ2 isozyme, except that it is not expressed in rat skeletal, cardiac and smooth muscles [33], in rat digestive organs [34], in hematopoietic cells and lymphoid tissues [35]. To date the only tissue where PLC δ2 was detected is bovine brain [5, 26]. The δ-type isozymes of PLC are the only phosphoinositide-specific phospholipases C found in lower eukaryotes such as yeast and slime molds [36, 37]. Based on this finding it might be speculated that δ-type isozymes are an archetype of other PLC isozymes, which evolved in higher eukaryotes.

REGULATION OF PLC δ ISOZYMES

In the action of PLC on a substrate localized in cell membrane two main steps can be distinguished, i.e., binding to the membrane surface and interaction with the substrate. Thus, the activity of PLC would depend on the factors that modulate association of the enzyme with the lipid membrane as well as on the factors that change interaction of PLC with a substrate. Studies on binding of PLC δ1 to lipid membrane showed that δ1 isozyme binds with high affinity to phospholipid vesicles containing PIP_2 and sphingomyelin [38, 39]. Phosphatidic acid was also reported to stimulate binding of myocardial PLC δ1 to plasma membrane [40]. The δ3 isozyme showed high specificity in binding to lipid membranes containing either PIP_2 or phosphatidic acid (Pawełczyk T. & Matecki A., unpublished). Experiments with the active proteolytic fragments of PLC δ1 [41] and further studies on the deletion mutants of δ1 isozyme revealed that the PH domain of PLC is required for interaction with the plasma membrane [11, 31, 42]. All eukaryotic PLC isozymes assayed *in vitro* required Ca^{2+} for activity. However, the δ isozymes are more sensitive to Ca^{2+} compared with the other PLC isozymes. The δ1 and δ3 isozymes when assayed with the substrate (PIP_2) located in detergent micelles are maximally activated by Ca^{2+} at a concentration range of 1–10 μM [9, 10, 24, 32]. PLC δ2 and PLC δ4 exhibited similar sensitivity to Ca^{2+} [6, 26]. The regulation of PLC δ1 *in vitro,* besides being dependent on calcium ions, critically depends on polyamines and phospholipids [43, 44]. Sphingomyelin is the most effective of the phospholipids tested for its ability to inhibit PLC δ1 [44, 45]. The δ1 isozyme is also inhibited by hexadecylphosphorylcholine and lysophospholipids showing antitumor activity [46]. The inhibition of PLC δ1 by sphingomyelin is promoted by spermine and Ca^{2+}, and is partially abolished by sphingosine, a breakdown product of sphingomyelin [16, 47]. Sphingosine and its homolog 4-hydroxysphingosine (phytosphingosine) activates PLC δ1 moderately in the liposome and detergent assay. The regulatory properties of PLC δ3 are different from those of PLC δ1. Under *in vitro* conditions polyamines and sphingosine inhibited PLC δ3 in all assays [9, 32]. When PLC δ3 acts on PIP_2 located in the phospholipid membrane, the Ca^{2+} concentration required to fully activate this isozyme is by one order of magnitude higher than that needed for PLC δ1 activation [32]. A study on the deletion mutants of PLC δ1 microinjected into Madin Darby canine kidney cells (MDCK) [14] suggested that PLC δ could be tethered to PIP_2-containing membranes *via* its PH domain in the absence of other signals. An increase in Ca^{2+} sufficient to activate PLC δ might therefore trigger its activation. It is possible that activation of PLC δ isozymes might occur secondarily to events leading to increases in Ca^{2+} concentration. However, the mechanism by which δ isozymes are coupled to membrane receptors remains unclear. The work on Chinese hamster ovary cells which overexpressed PLC δ1 indicated that thrombin-induced PLC δ1 activation is regulated *via* both G-protein and calcium [30]. Homma & Emori [48] reported that PLC-δ1 bound to a novel GTPase activating protein specific for protein-RhoA. It has been proposed that PLC δ1 activation occurs downstream of RhoA activation. Other findings on the linking of PLC-δ1 to cell surface indicate that $G_h\alpha$ protein that also possesses tissue transglutaminase activity, binds and activates PLC δ1 [49, 50]. It has also been reported that G_h protein is associated with agonist-stimulated α1-adrenergic receptor [51]. Thus, $G_h\alpha$ might represent a protein that directly couples PLC δ1 to this receptor.

RELATION OF PLC δ TO HUMAN DISEASE

In studies on human essential hypertension, spontaneously hypertensive rats are used as a

model. These studies have shown that in the aortas of these rats the activity of PLC $\delta 1$ is higher than in age-matched normotensive rats, whereas other PLC isozymes are unchanged [52]. Moreover, increased activity of PLC $\delta 1$ in aortas of hypersensitive rats correlates with changes in phospholipid composition of their aortas [53]. A study on PLC distribution in the kidney of these rats showed that the inner medullary concentration of PLC $\delta 1$ was significantly lower compared to that in normotensive rats [54]. These results suggest that PLC $\delta 1$ might play an important role in development of hypertension. Immunocytochemical methods used to established the distribution pattern of PLC isozymes in several human neurodegenerative diseases showed abnormal localization of PLC δ in patients' brains. PLC $\delta 1$ is abnormally accumulated in neurofibrillary tangles, the neurites surrounding senile plaque cores, and neurophil threads in Alzheimer brains [55–57]. Western blot analysis showed that in the brains of patients with Alzheimer disease the PLC $\delta 1$ concentration was significantly higher in the cytosolic fraction, and lower in the membrane fraction of cortical tissues. The changes in PLC $\delta 1$ localization in the brains of Alzheimer disease patients are associated with lowered specific activity of PLC $\delta 1$ [58]. The abnormal association of PLC $\delta 1$ with the filamentous inclusions in Pick's disease, progressive supranuclear palsy and diffuse Lewy body disease was also reported [59]. Examination of a series of human colon carcinomas revealed an elevated level of PLC $\gamma 1$ protein and decreased level of PLC $\delta 1$, as compared with the level of these enzymes in paired adjacent normal tissues [60]. Lowered expression of PLC $\delta 1$ was also reported in rat colon neoplasms induced by methylazoxymethanol [61].

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