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Minireview

Multiple roles of pleckstrin homology domains in phospholipase Cβ function

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Abstract Since their discovery almost 10 years ago pleckstrin homology (PH) domains have been identified in a wide variety of proteins. Here, we focus on two proteins whose PH domains play a defined functional role, phospholipase C (PLC)-β_2 and PLCδ_1. While the PH domains of both proteins are responsible for membrane targeting, their specificity of membrane binding drastically differs. However, in both these proteins the PH domains work to modulate the activity of their catalytic core upon interaction with either phosphoinositol lipids or G protein activators. These observations show that these PH domains are not simply binding sites tethered onto their host enzyme but are intimately associated with their catalytic core. This property may be true for other PH domains.

Key words: Pleckstrin homology domain; Phospholipase C; G protein activation; Membrane binding

1. Introduction to the phospholipase Cβ signaling system

When certain hormones, ions or neurotransmitters bind to their cognate heptahelical transmembrane receptor, these receptors catalyze the exchange of GTP for GDP on Gα subunits of heterotrimeric G proteins. The presence of GTP in the Gα binding site greatly reduces its affinity for Gβγ subunits which then allows both the Gα and Gβγ subunits to interact with a number of intracellular effectors. One of these effectors is phosphoinositol-specific phospholipase Cβ (PLCβ) (for reviews see [1,2]).

PLC enzymes play a key role in signal transduction by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PIP_2) which is found in cell membranes at low concentrations. The products of PIP_2 hydrolysis are the two second messengers inositol 1,4,5-trisphosphate and diacylglycerol which initiate the release of Ca^{2+} from intracellular stores and activate protein kinase C (see Fig. 1). The PLCβ family is one of four known families of mammalian PLCs (PLCβ, -γ, -δ and -ε) that differ in their structural organization and regulation (see [1,2]. All of the PLCs have multiple cellular regulators, but only the PLCβ enzymes are regulated by heterotrimeric G proteins. PLCγ enzymes are regulated by receptor and non-receptor tyrosine kinases, while protein regulators of PLCδ include RhoGAP and α_H and the newly discovered PLCε enzymes, which contain a GTP exchange factor and two RAS binding domains, is regulated by RAS [3,4].

There are four known types of PLCβs (β_1–β_4) which differ in their tissue distribution and their specific activity [1,2]. PLCβs are activated by both members of the $G\alpha_q$ family of G proteins and by Gβγ subunits [5–9]. The affinity and efficacy of Gα and Gβγ stimulation varies with each PLCβ isozyme [10,11]. To get insight into the mechanism through which G proteins activate PLCβ, we have focused much of our work on PLCβ_2 which is regulated by both $G\alpha_q$ and Gβγ subunits and compared its properties to the non-G protein-regulated enzyme, PLCδ_1.

2. The function of PLCβ_2 domains

PLCs are modular proteins consisting of a catalytic domain with several regulatory domains. The domain organization of PLCβ and PLCδ is shown in Fig. 2. Located on the N-terminus is a pleckstrin homology (PH) domain whose function is discussed in detail below. This region is followed by four elongation factor (EF) hands which do not appear to be Ca^{2+}-sensitive but may simply function as a flexible linker to the catalytic X-Y domain [12]. The catalytic domain is in the form of alternating α and β strands and is highly conserved among mammalian PLCs. Within the catalytic domain is an insertion region that is distinctive for each PLC family. The insertion region is unresolved in structural studies of PLCδ, and its functional roles both in PLCδ and in PLCβ are completely unknown; however, in PLCγ this region contains several domain modules that inhibit the basal activity of the enzyme until association with activated tyrosine kinase receptors. The catalytic region is followed by a C2 domain, which functions as a Ca^{2+}-dependent membrane binding anchor in PLCδ [13] whereas in PLCβ this region is responsible for strong and specific binding to activated $G\alpha_q$ subunits [14]. The PLCβ isozymes are distinguished by a ~400 residue C-terminal tail which is absolutely required for activation by α_q subunits [15,16].

While the crystal structure of an entire mammalian PLC has yet to be solved, the C-terminal extension of PLCβ [17], the PH domain of PLCδ [18], the catalytic domain containing two EF hands, the X-Y domain and the C2 domain of PLCδ have been solved [12]. The manner in which these separate

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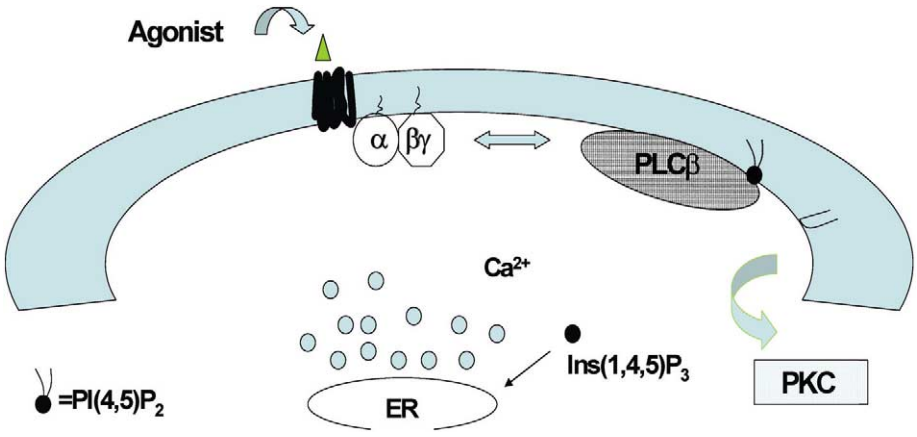

Fig. 1. Cartoon of the G protein–PLCβ signaling system. See Section 1 for details.

domains interact to regulate enzymatic activity is still unresolved.

3. Overview of PH domains

PH domains are small, modular structural domains consisting of ~120 residues that may be in as many as 252 proteins (for review see [19,20]). These domains were first identified in the N- and C-terminal regions of the protein pleckstrin. Their identification was striking since these domain have little sequence identity (i.e. only one conserved residue). Despite this lack of sequence identity, the predicted structure of these domains as well as the structure of the 13 PH domains which have been solved all show the same fold. The domain consists of seven β-strands with intervening loops of variable lengths. Three of the strands are parallel and orthogonal to the other four to form a β-sandwich with a closely packed interior. One end of the β-sandwich is closed off by a C-terminal α-helix (see Fig. 3). A characteristic that appears to be general for PH domains is that they are electrically polarized and this polarized nature may promote their association to target substrates.

The functional roles of PH domains may be as diverse as their sequence. Once thought to serve as an inositol phosphate binding domain, it now seems that only a few bind to phosphoinositol lipids or other lipids with strong specificity, and this lipid specificity is obtained by interactions with the variable loop regions on the N-terminal side rather than the conserved structural elements (see [20] and references therein). Another proposed function of PH domains is their specific binding to WD40 proteins which include Gβ subunits and this type of binding has been shown for several types of PH domains. It has been suggested that the C-terminal α-helix and the coextensive region of PH domains is responsible for interaction with the blades of WD40 proteins and many constructs of PH domains have included the ~20 residues following the α-helix, but this region does not appear to be part of the PH domain fold (see [19]). Thus, from a large body of work, we can draw the overall conclusion that the PH domain structure serves as a scaffold whose loop regions and extensions have evolved to meet the specific needs of the host protein. In the following review of the PH domains of PLCs, we describe how these domains not only serve as a ligand binding module, but make critical contact with catalytic regions of the protein to regulate enzyme activation.

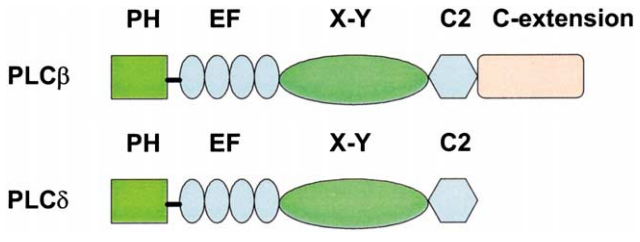

Fig. 2. Schematic diagram of the domain structure of PLCβ and PLCδ.

4. Contrasting the binding behaviors of the PH domains of PLCβ and PLCδ

One possible function of PH domains came from studies of PLCδ. It was known that PLCδ would only bind strongly to lipid bilayers if its substrate, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), was present and this $PI(4,5)P_2$-specific binding only occurred if the N-terminus of PLCδ, which was later to be identified as the PH domain, was intact [21]. These observations led to the idea that PLCδ has two $PI(4,5)P_2$ binding sites, one in the catalytic region and one in the PH domain. This second $PI(4,5)P_2$ binding site serves to keep the enzyme bound to the membrane surface to processively catalyze substrate. When the membrane concentration of $PI(4,5)P_2$ becomes low, and the level of aqueous product ($I(1,4,5)P_3$) becomes high, the PH-PLCδ will bind to product and catalysis will cease. The crystal structure of PLC-PHδ with bound $I(1,4,5)P_3$ that followed this model showed that specificity is due to a series of hydrogen bonds as well as electrostatic interactions [18].

In contrast to PLCδ, $PLC\beta_{1-3}$ enzymes will bind strongly to membranes with little specificity [22]. Presumably, this non-specific nature keeps the enzyme bound to the membrane to promote association with membrane-bound G protein subunits. Since removal of the ~400 residue C-terminal region reduces the amount of enzyme associated with the membrane fractions, the non-specific binding was attributed to the highly charged C-terminal extension [23]. However, preparing the purified isolated PH domains of $PLC\beta_1$ and -β_2 and measuring their ability to bind to various membrane substrates showed that these modules also bound strongly and non-spe-

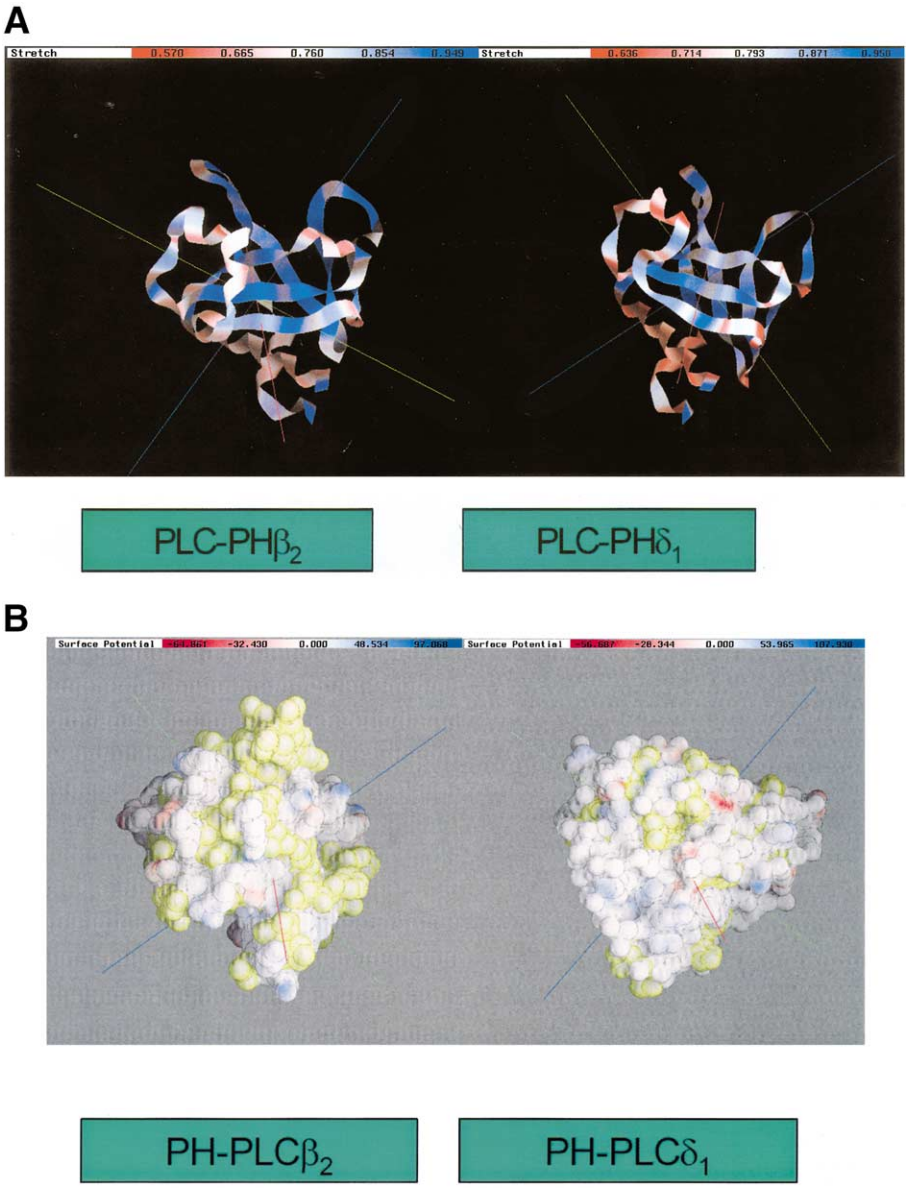

Fig. 3. A: Comparison of a model of the PH domain of PLCβ$_2$ (see text) as compared to PH-PLCδ$_1$ where the blue represents positive surface potential and the red represents negative surface potential. B: Comparison of the potential of the PH domains of PLCβ$_2$ and PLCδ$_1$ in which the potentials are imported into a transparent surface. Hydrophobic residues are colored in yellow.

cifically to membranes [24]. Thus, like PLCδ, the PHβ domains direct membrane binding of their host proteins.

Since the PH domains of several enzymes including the β-adrenergic receptor kinase bound to Gβγ subunits in pull down assays, and since PLCβ are Gβγ effectors, it was likely that the PH domains of PLCβ$_2$ and -δ$_1$ would serve as docking sites for Gβγ subunits. Measurements of these associations on membrane surfaces showed that PH-PLCβ$_2$ binds Gβγ subunits strongly with an affinity approximately five-fold higher than PH-PLCδ$_1$, which is not activated by Gβγ subunits [24]. These affinities directly correspond to the affinities of the whole proteins indicating that the PH domain is the primary binding site of Gβγ subunits. The difference in Gβγ affinity between PLCβ and PLCδ does not completely explain the differences in activation since PLCδ is not activated by Gβγ subunits even at concentrations where the two should be completely bound.

5. Model of the PH domain of PLCβ$_2$

We have attempted to understand this strong and non-specific membrane binding behavior of the rat PH-PLCβ$_2$ used here by constructing theoretical models based on the crystal structure of the PH domain of PLCδ. This model, shown in Fig. 3A, was obtained using 3D Jigsaw Comparative Modeling Server, and compares well with the model presented by Singh and Murray [25]. Our alignment gives a sequence identity between PH-PLCδ$_1$ template and PH-PLCβ$_2$ of 10% for the PLCβ$_2$ region 6–135. The model predicts a secondary structure identity of 82% and shows that PLCβ$_2$, like PLCδ$_1$, is also electrically polarized. This polarization is surprising when one considers that PH-PLCβ$_2$ will bind strongly to electrically neutral membranes with a similar affinity as negatively charged ones. However, association with neutral membranes may be mediated through the many non-

polar groups on the surface of the PH-PLCβ$_2$ domain (see below).

In Fig. 3B we show the electrostatic surface structure obtained using the programs GRASP. The potential is mapped onto a transparent surface in order to view the hydrophobic residue atoms which are colored in yellow. In terms of content, PH-PLCβ$_2$ contains ~10 more residues that are considered hydrophobic as compared to PH-PLCδ$_1$ and so we expect the predicted PH-PLCβ$_2$ structure to have a more nonpolar character. We find that this model predicts several hydrophobic patches on the surface of PH-PLCβ$_2$. These patches are expected to participate in docking to electrically neutral membrane surfaces, docking to Gβγ subunits or docking to the catalytic core of the enzyme.

6. Role of the PH domains of PLCβ and PLCδ in enzymatic function

While it was clear that the PH domain of PLCβ$_2$ acted to dock the enzyme to the lipid membrane as well as to Gβγ subunits, it was not clear of its role, if any, of modulating the activity of the enzyme. To address this question, our laboratory constructed a chimera composed of the PH domain of PLCβ$_2$ and the remaining portion of PLCδ$_1$ called PHβ$_2$-PLCδ [26]. As expected, this protein showed the same nonspecific membrane binding behavior and Gβγ affinity as PLCβ$_2$. However, replacing the PHδ for PHβ caused the high catalytic activity of PLCδ to undergo a large reduction to a level identical to PLCβ$_2$ (k_{cat} apparent = 140 s^{-1} for PLCδ and 2 s^{-1} for PHβ-PLCδ and PLCβ$_2$). Most importantly, this protein was activated by Gβγ subunits with an identical profile as PLCβ$_2$. Thus, the PH of PLCβ$_2$ confers G protein activation to the non-G protein-regulated enzyme, PLCδ, and similarly, residues in the PH domain of PLCβ$_2$ transmit Gβγ binding which then results in catalytic activation.

Several studies have implied that the PH domain of PLCδ directly modulates catalytic activity. Studies by Roberts and coworkers suggest that activation of PLCδ occurs upon binding to the membrane surface which is mediated by the PH domain [27]. However, activation by the PH domain is not simply due to tethering because mutations in the PH domain that affect PI(4,5)P$_2$ binding result in activation. Moreover, it has been found that PI(4,5)P$_2$ is an activator of PLCδ [28].

While it may seem that the PH domains of PLCβ$_2$ and -δ$_1$ function to transmit the binding of activator (i.e. Gβγ or PI(4,5)P$_2$ for PLCβ$_2$ and -δ$_1$, respectively) to the catalytic domain, recent work suggests that the PH domain is intimately involved with the catalytic region. Using a chimera in which the PH domain of PLCδ was swapped into the catalytic domain of PLCβ$_2$, it was found that the PI(4,5)P$_2$ activation behavior of PLCδ was preserved and the Gβγ stimulation abolished. Surprisingly, the enzyme was no longer activated by Gα$_q$(GTPγS) (Guo and Scarlata, submitted). This observation was unexpected because both the docking site and the region that transmits activation are sequentially far removed from the PH domain and implies that the PH domain is the main regulatory region for G protein activation.

The mechanism through which the binding of either PI(4,5)P$_2$ or Gβγ activators to the PH domains of PLCδ and PLCβ, respectively, transmits activation to the catalytic site is unclear. While the results described above suggest a direct interaction between the PH domain and the catalytic site, this need not be the case, and it is possible that activator binding leads to a change in the orientation of the domain with the membrane surface or catalytic core to produce activation. Crystallographic studies of PLCδ$_1$ in the presence of I(1,4,5)P$_3$ product showed the PH domain to be unresolved suggesting a loose association with the catalytic core [12]. Thus, it is possible that the binding of activator to the PH domain proceeds by removing some inhibitory interaction of the PH domain with the catalytic core. We note that the model of PH-PLCβ$_2$ in Fig. 3 shows several hydrophobic patches as compared to PH-PLCδ$_1$ and offers the possibility that close contact with the core protein occurs in the unactivated state.

Whether or not activation of the catalytic core occurs directly or indirectly by activator binding to the PH domain of PLC, it is clear from the chimera studies that the region in the catalytic core that produces activation must be conserved in the two PLCs. A body of work has pinpointed a helix containing residues Glu574–Lys583 which lies above the catalytic site of PLCβ$_2$ as playing a key role in Gβγ activation [29,30]. Since both PLCβ and PLCδ PH domains can activate the catalytic core of both proteins, there must also be some homologous regions in the two PH domains that makes contact with the protein. These homologous regions have yet to be identified.

7. Conclusions

Rather than a simple docking domains tethered onto their hosts, the PH domains of PLCs are directly liked to the regulation of their catalytic core. While these domains serve to dock multiple elements, such as protein partners and membrane lipids, they may also dock site in the protein matrix to directly or indirectly affect catalytic activity. This ability of bringing together several elements may underlie the prevalence of these domains is so many proteins.

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