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Stimulation of Phospholipase Cβ by Membrane Interactions, Interdomain Movmement, and G protein Binding - *How Many Ways Can You Activate an Enzyme?*

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Abstract

Signaling proteins are usually composed of one or more conserved structural domains. These domains are usually regulatory in nature by binding to specific activators or effectors, or species that regulate cellular location, etc. Inositol-specific mammalian phospholipase C (PLC) enzymes are multidomain proteins whose activities are controlled by regulators, such as G proteins, as well as membrane interactions. One of these domains has been found to bind membranes, regulators, and activate the catalytic region. The recently solved structure of a major region of PLC-β2 together with the structure of PLC-δ1 and a wealth of biochemical studies poises the system towards an understanding of the mechanism through which their regulations occurs.

Keywords

PLC; Gβγ; PH domain

1. Introduction

Almost all known signaling proteins consist one or more conserved structural domains attached to an active site that in some way modulate the catalytic activity. An example of this is phosphatidylinositol specific mammalian phospholipase C (PI-PLC or here, termed PLC). PLCs are Ca^{2+}-dependent enzymes that catalyze the hydrolysis of phosphatidylinositol 4,5 bisphosphate ($PI(4,5)P_2$) to yield insoitiol 1,4,5 trisphosphate ($Ins(1,4,5)P_3$) and diaceylglycerol (DAG), which are important secondary cellular messengers. $Ins(1,4,5)P_3$ binds to its receptor in the endoplasmic reticulum to open Ca^{2+} channels which increases the intracellular level of Ca^{2+} and activates a host of calcium-sensitive enzymes, such as protein kinase C (PKC) which is also co-activated by the appearance of DAG (Figure 1).

PLCs can be found at all stages in evolution. The simplest bacterial PLCs consist solely of the catalytic lipase domain which requires Ca^{2+} for activity. As species progressed and families of PLCs began to grow, PLCs became larger and more complex as regulatory domains were added. Presently, there are six major known families of mammalian PLCs (-β, -γ [1], -δ, ε [2], ζ [3], -η [4]) which consist of at least 13 isoforms that differ in structural organization,

regulation, activation and tissue distribution. This review focuses on mechanism of activation of PLC-βs and PLC-δs.

PLC-δs can be found in all tissue types. Like all PLCs, PLC-δs require Ca^{2+} for activity, but unlike other mammalian PLCs, PLC-δ enzymes are inactive at basal levels of Ca^{2+}. The increase in intracellular Ca^{2+} resulting from the activation of other PLCs will activate PLC-δ, making this enzyme downstream of other PLCs in signaling pathways. As detailed below, some protein regulators change the level of Ca^{2+} required for PLC-δ activation. PLC-δ1 was the first mammalian PLCs to have high resolution structures available [5,6] and, since it can be expressed easily, a great deal of biophysical information is available (see [7]) allowing us to contrast its properties with other, closely related PLCs.

PLC-βs are the major effectors of the $G\alpha_q$ family of heterotrimeric G proteins (Figure 1). This family is coupled to receptors that binds ligands such as angiotensin II, catecholamines, endothelin 1 and prostaglandin $F_{2\alpha}$., bradykinin, etc.. There are four known family members of PLC-β that differ in their response to G proteins. All PLC-β are strongly activated by GTP-bound $G\alpha_q$ in the following order :β 1, β4 > β3 > β2 [8]. Additionally, PLC-β2 and PLC-β3 are activated by Gβγ subunits [9,10]. The mechanism of activation by G protein subunits is not well understood but the recent structure of PLC-β2 complexed with the small GTPase Rac2 may offer some clues [11], as discussed below. The following sections describe the role that individual domains of PLC-δ1 and PLC-β2 play in regulating enzyme activity.

2. Structural Organization of PLC-β and PLC-δ and Function of Domains

PLCs contain an N-terminal pleckstrin homology (PH) domain, followed by four EF hands, a catalytic domain (X/Y domain) and a C2 domain **(**Figure 2). Additionally, PLC-βs are distinguished from PLC-δs by a 400 residue C-terminal extension downstream of the C2 domain that plays a role in G protein activation and nuclear localization, as discussed later in this review.

2.1. Catalytic domain and mechanism

The catalytic domains of PLCs are highly conserved from bacteria through mammals [12]. The domain is composed of two regions, X and Y that are linked to form two halves of the catalytic site in a distorted Triose Phosphate Isomerase (TIM) α/ β barrel configuration. The active site is at the end of the barrel (Figure 4) and appears as a shallow cleft with the remainder of the domain appearing as a dome covering the substrate. Ellis and coworkers have defined the identity of the residues that are involved in giving PLC-δ1 a preference for $PI(4,5)P_2$ and in binding Ca^{2+} which is a required catalytic cofactor. Their work demonstrates the crucial role of His-311 and His-356 for the acid/base catalytic process [13] . Interestingly, at one end of the PLC-δ1 active site is a convex ridge of hydrophobic residues that penetrate into the membrane to allow the catalytic site to access substrate. This penetration explains the decreased activity of PLC-δ1 on membranes with high lipid packing [14,15]. A similar but smaller rim is found in prokaryotic enzymes [12,16]. Also noteworthy is the key role of water in directing contacts which, if the enzyme does penetrate the membrane surface, must be part of the hydration layer or part of the enzyme hydration boundary water molecules.

In general, PLCs catalyze the hydrolysis of all PI lipids except PI(3)P (see [17]). Mammalian PLCs generally prefer $PI(4,5)P_2$ due to stabilizing interactions of the 4- and 5- phosphate with specific residues (e.g. Ser 522, Lys 438, Lys 440 and Arg549) and regions of the Y domain of PLC-δ1 [18]) but PI(4)P and PI lipids are also hydrolyzed. As mentioned, the catalytic domains of PLCs are highly homologous and a comparison of the domains of PLC-β2 and PLC-δ1 structures gives a RMS of 0.82 Å.

As shown in Figure 4, PLC-δ1 and -β2 hydrolyse PI(4,5)P_2 into Ins(1,4,5)P_3 and diacylglycerol in two steps [18–20]. The first, (i.e. the phosphotransferase step) is initiated by an intramolecular attack of the inositol 2-OH group on the phosphodiester phosphate of PI(4,5)P_2 One molecule of diacylglycerol and Ins(1:2-cyclic 4,5)P_3 are the final products in the reaction catalyzed by bacterial enzymes [21]. Additionally, the mammalian PLCs are capable of holding the cyclic product in the active site long enough to allow for hydrolysis by water producing a linear product (i.e. the phosphodiesterase step) [20]. The penetration of the hydrophobic ridge into the membrane surface may help to stabilize and retain PI(4,5)P_2 and subsequent cyclic intermediates in the active site. While PLC-γ and PLC-δ generate both linear and cyclic InsP_3 products, whose relative amounts depends on the reaction conditions (pH, temperature, substrate, Ca^{2+} concentration) [22], PLC-β_2 only produces the linear product suggesting that in PLC-β_2, cyclic InsP_3 is bound to the catalytic site long enough to allow its complete conversion [19].

Despite their strong conservation in the catalytic domains, mammalian PLC exhibit a sequence of high variability linking the X and Y domains, as shown in the Figure 4 for PLC-δ_1 and PLC-β_2. These linkers are not or only partially resolved in the crystal structures [5,6] and are susceptible to proteolysis [23] suggesting high flexibility. The number of charged amino acids, especially the long stretch of negatively charged residues, in PLC-β2 is striking. Partial resolution of this linker in PLC-β2 shows that it occludes the catalytic site and impairs access to PI(4,5)P_2. In line with this, it has been reported that the removal of the linker generates a PLC-β_2 with a higher basal activity suggesting that the linker exerts an inhibitory effect on this enzyme [24,25] . However, neither Gβγ nor Gα_q activate the PLC-β2 via this linker. Indeed, this region could be required for the regulation of the enzyme by other, unknown cellular factors. We speculate that the linker inhibits the enzyme until it is displaced by electrostatic repulsion upon membrane binding. Interestingly, it was shown that a peptide corresponding to the segment 448–464 of PLC-β2, localized in the X region, and containing residues K461 and K463 which bind ins(1,4,5)P_3, activates the enzyme [26]. The structure of PLC-β2 shows that the resolved portion of the X/Y linker is partially in contact with this segment [11]. This suggests the possibility that the activation occurs when the peptide competes with the catalytic site to bind to the X/Y linker, triggering the unmasking of the catalytic site. In support of this idea, Roberts and collaborators point out that initiation of ligand binding to the active site could shift the PLC-δ1 to more active form of the enzyme, possibly through a conformational change involving the X/Y linker [20]. In the case of PLC-γ, the X/Y linker contains a split PH-domain, two SH2 domains, and SH3 domain which, by binding specifically to specific lipids and adaptors, can induce an intramolecular rearrangement gathering the separated X and Y region and reconstituting a fully-active catalytic site [27].

2.2. Pleckstrin Homology (PH) domains

The PH domain of PLC-δ1, which encompasses the first ~130 residues, was one of the first PH domains to be crystallized [5]. Its structure constitutes a canonical PH domain fold: a seven-stranded β-sandwich topped off by a C-terminal α-helix (Figure 3). Although they all adopt the same structure, PH domains have only one conserved residue: a Trp in the C-terminal helix whose side chain serves as a template to nucleate the collapse of hydrophobic residues, thus giving the interior of PH domains a dense protein core [28]. This core serves to properly display specific `functional' residues of the solvent-accessible β/β loops between each β-strand. The sequences of these loops are highly variable and define the specificity of each kind of PH domain.

Functionally, many PH domains target particular lipids and membrane surfaces [28]. The PH domain of PLC-δ1 (PH-δ1) [5] was the first of these domain whose function was determined. It had been established earlier that PLC-δ1 bound strongly and specifically to membranes

containing $PI(4,5)P_2$ [29]. Rebecchi and coworkers then found that when they enzymatically cleaved the N-terminus, which was later identified to be a PH domain, the enzyme was still fully active, but it no longer bound $PI(4,5)P_2$- containing membranes [23]. The idea that the PH-δ1 was responsible for specific $PI(4,5)P_2$ binding was proven using the isolated PH domain [30,31]. Insight into the high affinity between the PH-δ1 domain and the polar head of $PI(4,5)P_2$ came from the crystal structure of this domain bound to $Ins(1,4,5)P_3$ [5] The overall domain is electrically polarized with a large positive lobe surrounding the binding site for $Ins(1,4,5)P_3$. Contacts between $Ins(1,4,5)P_3$ and PH-δ1 are as specific as any enzyme-substrate interaction and consist of several hydrogen bonds and ionic contacts mostly between residues in the β1/β2 and β3/β4 loops and the 4- and 5-phosphate groups of Ins(1,4,5)P3 (Figure 3).

PLC-δ1 has been demonstrated to display a scooting behavior in which the enzyme binds to the membrane and hydrolyzes numerous substrates before returning to the aqueous phase [23,32]. The observation that the PH domain binds to $PI(4,5)P_2$ with an affinity ~1000 fold stronger than that of the catalytic site explains this interfacial enzymatic behavior. The high affinity site of PH domain (KD ~ 1 μM) serves to anchor the enzyme to membranes containing $PI(4,5)P_2$, allowing for processive catalysis by the lower affinity catalytic site (K_D > 0.1 μM) [31,33]. It is notable that $Ins(1,4,5)P_3$ binds more strongly to PH-δ1 than $PI(4,5)P_2$, but since the effective interfacial concentration of $PI(4,5)P_2$ is very high at the membrane surface, the enzyme will remain bound to the membrane until the $Ins(1,4,5)P_3$ concentration in the aqueous phase is much higher [33,34]. Thus, by regulating the association of PLC-δ1 to membranes, the PH domain plays a key role in governing the activity of this enzyme. Moreso, the PH domain plays a key role in localizing it to substrate-containing membranes in cells [35].

Like PLC-δ1, it has been reported that PLC-β2 forms multiple contacts with lipid interface, leading to a processive mode of $PI(4,5)P_2$ hydrolysis [36]. However, in contrast to PLC-δ1, PLC-β2 as well as PLC-β1, bind to membranes with high affinity but little specificity, independent of $PI(4,5)P_2$ [37–39] . Their isolated PH domains are unable to bind to $Ins(1,4,5)P_3$ [33] but, together with the C-terminal region [38], allow PLC-β1 and PLC-β2 to interact non-specifically with membranes [40]. Structurally, the PH-β2 domain is highly homologous to that of PLC-δ1 (Figure 3) and is expected to possess a similar orientation on membrane [11]. However, the β5/β6 loop is longer than its counterpart in PH-δ1 as expected from homology modeling [41]. The lack of binding of PH-β2 to $Ins(1,4,5)P_3$ and $PI(4,5)P_2$ results from the absence of the residues used by the PH-δ1 to specifically clamp the phosphate groups of the inositol [11].

The molecular basis for the non-specific membrane avidity of PH-β2 is unclear. Since the PH-β2 interacts strongly with neutral membranes [40], it has been proposed that its high overall hydrophobicity could explain its anchorage to electrically neutral membranes [42]. Others speculate that the highly hydrophobic β3/β4 patch of PH-β2 could insert into the lipids [43] but this region constitutes the interface of the PH domain with the EF-hand region of PLC-β2. It is worth noting that the crystal structure of PLC- β2 suggests that its PH domain is not in contact with membranes [11], however, one could imagine, as believed for PLC-δ1, that some flexibility could allow for lipid binding. Theoretical models suggest that the isolated domain could adopt several orientations of low energy and point out possible membrane binding motifs based on hydrophobicity [41].

2.3. Elongation Factor (EF) Hands

PLCs contain 4 EF-hands that are connected to the PH domain by a short linker. Unlike the role of EF-hands in other host proteins, the EF-hands of PLCs do not undergo Ca^{2+} – dependent conformational changes. There are data suggesting that the EF-hands of PLC-δ1 bind specifically to fatty acids and that this binding stimulates the catalytic activity of the enzyme, implying that PLCs may be sensitive to the nature of the hydrocarbon chains [44,45]. Although

the crystal structure shows limited contact between the EF hands and the catalytic domain, their role in the regulation of the enzyme either directly or through interaction with other proteins or cofactors remains to be seen.

2.4. C2 Domain and C-terminal extension

Presently, we have information about both the structures and functions of the C2 domain domains of PLC-δ1 and PLC-β2. C2 domains, denoting the second constant region of PKC, span ~120 residues and are primarily compact sandwiches of two four stranded β sheet that serve as scaffolds for loops that carry out specific functions. The structure of the C2 domain of PLC-δ1 reveals the existence of up to three binding sites for calcium [46]. Although suppression of these binding sites does not impair the ability of PLC-δ1 to hydrolyze PI(4,5) P_2 [47]. It has been shown that PLC-δ1 exhibits a specific Ca^{2+}-dependant binding to PE-containing membranes through its C2 domain [48]. Since the C2 domain is almost integrated with the Y region, it is thought to assist in orienting the catalytic site of PLC-δ1 onto the membrane once bound through initial association by the PH domain (i.e. the “tether and fix” model).

In PLC-β2, the C2 domain binds weakly to membranes but it has a novel function in that it strongly and specifically binds to activated $G\alpha_q$ subunits [49]. This domain does not bind to $G\alpha_i$ or $G\beta\gamma$ subunits, and its interaction with $G\alpha_q$ in its GDP bound state is ~10 fold weaker than its binding to activated $G\alpha_q$.

As mentioned above, PLC-β enzymes are distinguished from other PLCs by a ~400 residue C-terminal extension. This region is absolutely required for activation by $G\alpha_q$ [24] and appears to serve other regulatory roles. It is noteworthy that the C-terminal domain also contains several phosphorylation sites that may regulate the extent of $G\alpha_q$ activation and possess in addition a nuclear localization signal [50]. Splice variants of PLC-βs differ in this region and in their cellular localization suggesting this region regulates transit into and out of the nucleus.

The structure of the isolated C-terminus of turkey PLC-β2 has been solved [51]. It is composed of three long helices forming a coiled-coil that dimerizes along its long axis in an anti-parallel orientation. This region, containing a high density of charged residues, is electrostatically polarized, and it is not surprising that it assists in membrane binding of PLC-β2 with the PH domain [52,53]. Mutations of interdomain residues in the isolated tails decreases the ability of the tails to compete with the native enzyme for activated $G\alpha_q$. [54]. However, the oligomerization state in solution and on membranes is unclear, and we, by fluorescence resonance energy transfer methods (Scarlata S., unpublished)., and others [36] have only been able to detect PLC-β2 dimers bound to membranes at high concentrations, although it has been reported that PLC-β1 and PLC-β3 are able to homodimerize [55].

3. Activation of PLC-δ and -β

3.2 Activators of PLC-δ1

Unlike PLC-βs which has well-established and important protein regulators, there have been several identified protein regulators for PLC-δ1 but none appear to significantly control its cellular activity (see [7] for review). Some regulators lower the level of Ca^{2+} needed for PLC-δ1 activation making the activity of this enzyme less reliant on the stimulation of other PLCs [56,57]. The mechanism of regulation is unknown.

It is possible that PLC-δ1 has a naturally occurring inhibitors, such has been found for PLC-δ4 [58]. An important class of PLC-δ1 inhibitors was found to be PLC-β2 and PLC-β3 [59]. It was shown that PLC-δ1 and PLC-β2 associate on membrane surfaces and that this association inhibits the activity of PLC-δ1, but not PLC-β2 [59]. PLC-δ1 bound to PLC-β2 can be displaced

by Gβγ subunits released upon cell stimulation. We speculate that inhibition of PLC-δ1 may prevent spurious or premature activate of this catalytically robust enzyme thus better controlling cellular Ca^{2+} levels.

3.3 Protein Activators of PLC-βs

The main cellular activators of PLC-β enzymes are Ras-like GTPase of the Rho family and heterotrimeric G proteins. Rac1, 2, 3, and to a lesser extent Cdc42Hs, were shown to activate PLC-β2 and β3 [60,61] by binding to their PH domains [11,61]. This binding was thought to activate the PLC-βs by recruiting them to the membrane surface [62]. In contrast, heterotrimeric G proteins activate PLC–βs without promoting its binding to membranes [37,39] and also without affecting the calcium dependence of its activity [10,37,38]. Instead, the strength of activation of PLC-β2 and -β3, at least by Gβγ subunits, appears to be directly proportional to the strength of association between the proteins [63]. We now wish to focus on the basis of the association and activation of PLC-βs by G proteins, and in particular Gβγ heterodimers since more information is available for this system.

4. Association of PLC-β2 by Gβγ

4.1 PLC-β2 binding site(s) in Gβγ

Numerous studies have mapped the regions of Gβγ interacting with PLC-β2. Gβ subunits have two domains, an N-terminal α-helix folded in a coiled-coil with the Gγ subunit and a seven-blade β-propeller structure (Figure 5) with each blade containing a WD motif [64]. By testing different mutants of Gβ subunits, Ford and coworkers found several key residues important for the activation of PLC-β2 in blades 1, 2 and 5 of the top side of the β-propeller [65] . Many of these regions interact with Gα subunits suggesting that the Gα and PLC-β2 binding regions on Gβ overlap. Thus, G protein activation must involve unmasking binding sites in Gβγ for PLC-β2. Buck and coworkers extended this work and found that a $G\beta\gamma_{86–105}$ peptide corresponding to a segment of the first blade can substitute for Gβγ and activate the enzyme even at saturating levels of Gβγ [66]. This peptide was therefore defined as the signal transfer region of Gβ. Extensive studies with other peptides identified a second signal transfer region ($G\beta_{42–54}$) in the seventh blade of Gβ, and revealed segments in the second, fifth and seventh blade that hold the Gβγ/PLC-β2 complex together during activation (Figure 5) [67,68]. In addition, it was found that the geranylgeranylated moiety attached to the C-terminal segment of the Gγ, which is proximal to the membrane and anchors Gβγ to lipids, binds to the PLC-β2 [69,70]. Moreso, Smrcka and colleagues, proposed that the 23–27 region of the N-terminal helix of the Gβ subunit was a second PLC-β binding site maintaining the enzyme outside the propeller in an inhibition state [71]. Thus, it appears that the top and the edge sides of the propeller is the region of Gβγ which binds to PLC-β and promotes activation. It is also noteworthy that the structure of Gβγ offers the possibility of secondary weaker interaction sites with PLCβ in addition to its primary high affinity site. However, the location of the Gβγ -binding site(s) on PLC-β2 are still unclear, and even more unclear is the mechanism of activation.

4.2 Gβγ binding site(s) in PLC-β2

From numerous studies over the past decade, it appears that there are probably two major binding sites for Gβγ in PLC-βs. We demonstrated *in vitro* that the PH domains of PLC-β1 and -β2 bind strongly to Gβγ heterodimers at the membrane surface [40]. In line with this, by swapping the PH domain of PLC-β2 into the remainder of PLC- δ1, we obtained a chimera that binds non-specifically to membranes like PLC-β2, and moreover, binds and is responsive to Gβγ subunits [72]. In two distinct studies, it was found that the N-terminal region of PLC-β3 encompassing the PH domain and a part of the EF-hands region binds to Gβγ [73] whereas it was reported that Gβγ helps the recruitment of a GFP-tagged PH-β1 on subcellular

membranes [74] Additionally, an independent series of studies located a solvent-accessible segment of nine residues (574–583 segment) in the Y region that is able to bind to Gβ [71, 75–77]. This segment, although partially-conserved in PLC-δ1, could govern the oscillation of the PLC-β2 between two distinct parts of Gβ subunit to be distal or proximal to the membrane surface [71].

All these results are compatible with the spatial proximity between the PH domain and the X/Y domain, as revealed recently [11]. For example, this idea also corroborates studies by Illenberger and collaborators using chimera of PLC-β1/PLC-β2 which show that both the PH and the catalytic domain of PLC-β2 are required for Gβγ - activation [78].

5. Constructing an activation model of PLC-β2 by Gβγ

For a long time, it was not clear how the PH-δ1 domain interacts with the three-domain structure of the remainder of PLC-δ1. The inability to solve the complete structure suggested a high degree of movement between the PH domain and the catalytic core and initiated numerous models of how the PH domain may contact the catalytic site [53]. Now that the structure of PLC-β2 is available, questions arise about the extent that the enzyme can “breathe”. Sondek and collaborators indicate that PLC-β2 is a rigid structure and suggest that the catalytic domain, but not the PH domain, interacts with membranes [11]. From this observation, they propose that PLC-β2 is activated by Gβγ through recruitment via the PH domain to membrane bringing the catalytic domain in contact with $PI(4,5)P_2$.

However, it has been shown that PLC-β2 has high intrinsic binding affinity and is already bound to membranes before activation by Gβγ and thus activation does not occur by promoting PLC-β translocation [37–39]. More striking is the identification of soluble fragments of Gβγ, $Gβ_{86–105}$ and $Gβ_{42–54}$, that are able to convey full activation of PLC-β2 [66,67,79]. Additionally, Gβγ has been shown to stimulate the phosphodiesterase step of the $PI(4,5)P_2$ hydrolysis reaction which is not dependent on membrane surfaces, and further biochemical studies have shown that in the presence of Gβγ subunits, product inhibition does not occur suggesting that activation is through an enhanced release of product [19]. These results suggest that binding of Gβγ subunits in some way promotes a change of its conformation that allows for activation.

Additionally, a series of studies by Smrcka and colleagues suggest that the X/Y domain oscillates between two positions with respect to Gβγ: one that is far from the membrane interacting with the N-terminal part of Gβ, and a second that is close to membrane and anchored to the propeller. In this latter configuration, it is likely that in addition to be positioned close to the membrane, PLC-β2 may experience conformational changes induced by Gβγ as pointed out by the existence of signal transfer peptides.

5.1. Role of the PH domain in PLC-δ1 and PLC-β2 Activation

As mentioned, we have found that the PH domain of PLC-β2 confers activation to the catalytic core. Similarly, by monitoring the activity of PLC-δ1 towards PI substrates, Lomasney and coworkers found that it becomes activated when its PH domain binds specifically to $PI(4,5)P_2$, suggesting that stimulation of this enzyme is also conferred by its PH domain [32]. This activation had been previously missed since the activator, $PI(4,5)P_2$, was the same as the substrate. It should be noted that the PH-δ1 is not a rigid lipid-binding domain since a slight conformational change, involving a small amphipathic helix of β5/β6 loop occurs upon binding to $PI(4,5)P_2$ and this change is regulated by the phosphatidylserine content in membranes [80,81]. Roberts and collaborators also suggest that the PH domain may have an allosteric role in PLC-δ1 activity [20]. It is thus likely that signal transfer occurs through a conformational change that is initiated when the PH domain of PLC-δ1 binds $PI(4,5)P_2$. Studies using a chimera

containing the PH domain of PLC-δ1 and the remainder of PLC-β2 indicate a $PI(4,5)P_2$-depending membrane binding and activation by $PI(4,5)P_2$ and not by Gβγ subunits [53]. This observation suggests that PH-β2 and –δ1 domains contact a conserved region in the catalytic domains of PLC-β2 and –δ1 in a similar way allowing their activators, $PI(4,5)P_2$ or Gβγ to be transferred when the PH domains are swapped. In line with this, the $Gβ_{86–105}$ peptide can activate PLC-δ1 as well as PLC-β2 [41] suggesting also that the catalytic regions of the two enzymes have a conserved activation site. Of note is the observation that the PH-β2 and –δ1 domains can adopt the same orientation in their host enzyme relative to the plane of the membrane [11], explaining the ability to swap the PH domain of PLC-β2 and –δ1 and change their responsiveness to activators.

Studies have been carried out to determine the regions of the PH-β2 domain that are responsible for Gβγ activation. Comparing a model structure of PH-β2 with the known structure of PH-δ1 suggests that the β5/β6 loop, which is longer than in PH-δ1, (Figure 3) could act as a regulatory domain for activation by Gβγ [41]. A peptide corresponding to this loop ($PHβ_{71–88}$) activates the enzyme, and this activation is competitive with Gβγ activation. The structure of PLC-β2 reveals that the β5/β6 loop does not belong to the surface that is tightly packed with the EF-hands and the catalytic regions, or to the hydrophobic ridge interacting with Rac2. Since the binding sites for Rac and Gβγ are most likely distinct, then it is probably that this region is involved in Gβγ -dependent activation of PLC-β2.

The ability of the peptide corresponding to $PHβ_{71–88}$ to activate the enzyme was unexpected. In an effort to understand this behavior, it was noted that this peptide contained several positively charged residues and was found to bind strongly to negatively charged membrane surfaces. It was observed that the presence of peptide $PHβ_{71–88}$ did not affect the binding of the whole protein but decreased the membrane binding of the catalytic domain. These results suggest that the presence of peptide may work to position the catalytic site on the membrane. Additional FRET studies monitoring changes in the distance between the PH and catalytic domains showed that addition of Gβγ altered the position of the domains in respect to each other. Taken together, these results suggest that activation of PLCs through the PH domain may involve interdomain movement that places the enzyme on the membrane surface in a productive way, perhaps through displacement of a region of the X/Y linker. We know that the movement must be small since the volume change that accompanies Gβγ is small [82].

5.2 Role of the Membrane Surface in Activation

While the membrane surface plays a major role in PLC activation, the multidomain organization of the PLC which contains several putative low and high-affinity membrane binding sites, makes it difficult to link membrane composition and phospholipase activity. For example, the effect of membrane components on PLC activity other than PI lipids appear to be specific. Anionic lipids such as PS will increase PLC-δ1 activity [32,48] through specific interactions between PS and the C2 domain of the phospholipase [48], and interactions between the EF hands and free fatty acids also stimulate PLC-δ1 activity region [44]. Specific interactions PA and the C-terminal tail of PLC-β1 will promote its activation [83].

Some of these membrane components affect PLC activity through changing the presentation of the substrate. It also is known that surface pressure is a key parameter governing the activity of the PLC-β and PLC-δ[15,84]. Mixing $PI(4,5)P_2$ with non-substrate lipids as PC or PS in place of detergent diminishes the PLC-β2 activity, since the scissile bond of $PI(4,5)P_2$ is less accessible [36]. Sphingomyelin may inhibit PLC-δ1 through a similar mechanism [85]. DAG, a hexagonal phase forming lipid and the product of the PI(4,5)P2 hydrolysis, will limit activity above a certain concentrations in some enzymatic assays [86]. Interestingly, PE lipids, which have smaller head groups as compared to PC and allow for better penetration of the enzyme into the membrane surface increases slightly the activity of PLC-δ1 [32] and allows PLC-β2

to be activated by $G\beta_{86–105}$ [41]. Moreso, a recent study shows that the PS content of a membrane influences conformational changes of PH-δ1 upon binding to $PI(4,5)P_2$ [81]. Thus, membrane organization as well as lipid packing are parameters which have to be examined in the future to better understand the activation of PLC-δ and –β.

Conclusions

Activation of PLC-δ and PLC-β appear to involve a dizzying array of variables including multiple interactions between domains of the protein, multiple interactions with protein regulators, multiple interactions with the membrane surface, and the composition and packing of the membrane surface itself. The activation processes itself seems to involve several interdependent and independent steps. While attempts to isolate these effects using minimalist strategies have been productive, it is now time to combine these many experimental studies with theoretical methods to develop unified models of activation. It is also interesting to speculate the evolutionary reasons for these multiple forms of activation and which form may be utilized under particular circumstances.

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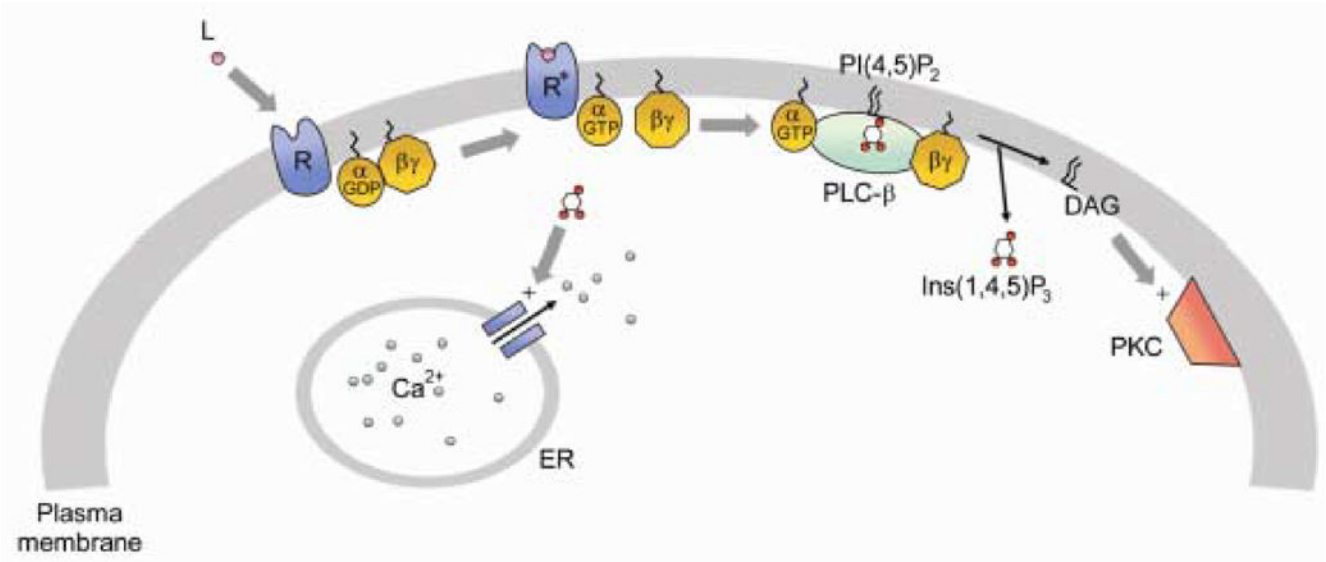

Figure 1. Cartoon on the G protein-PLCβ signaling system

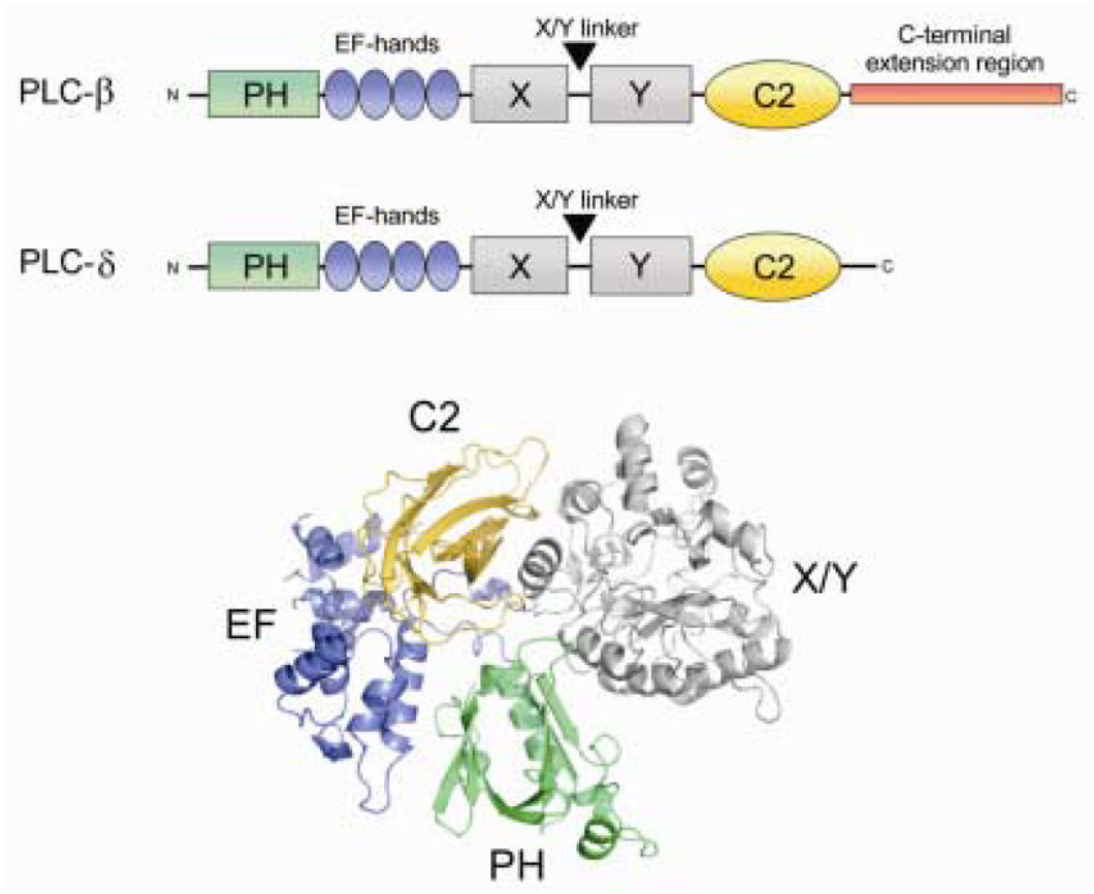

Figure 2. Organization of PLC-β and –δ domains

Top – Schematic diagram showing the domains organization of PLC-β and –δ. *Bottom* - The structure of the human PLC-β2 represented in ribbon (PDB entry : 2FJU) showing the organization of the different domains.

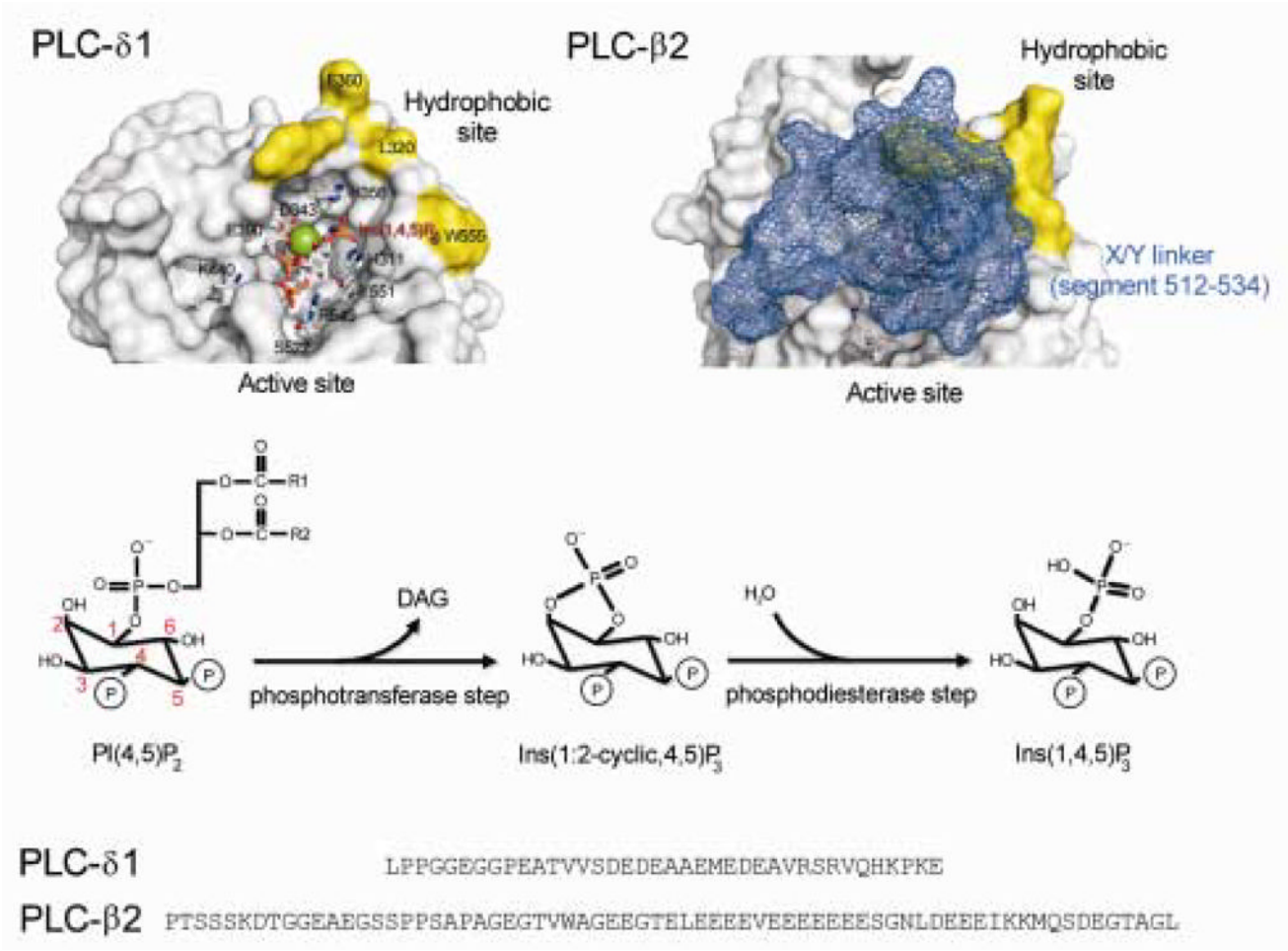

Figure 3. Comparison of the structure of the PH domains of the PLC-δ1 and –β2

Top left – The structure of rat PH-δ1 (PDB entry :1MAI [5]) showing the hydrogen-bonds between the Ins(1,4,5)P3, water molecules and the K30, K32, W36, R40, E54, S55, R56, K57 and T107 residues are represented in green. *Top right* - The structure of the human PH-β2 (PDB entry : 2FJU [11]) showing the long β5/β6 loop (highlighted in green. *Bottom* - The structural alignment of PH-δ1 and PH-β2 sequences showing the absence of the Ins(1,4,5)P_3 binding residues and the longer β5/β6 loop of PH-β2.

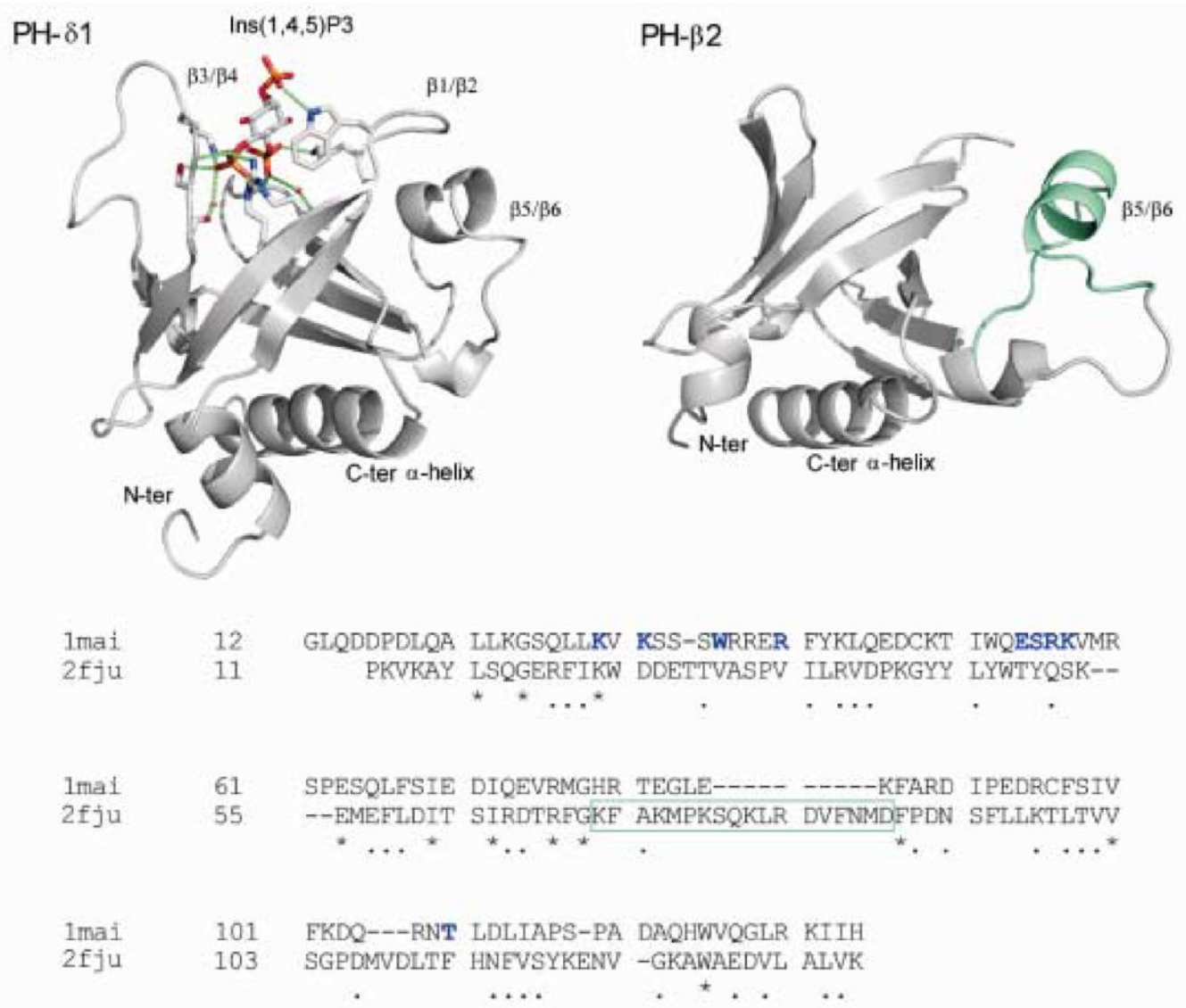

Figure 4. Catalytic X/Y domain of the PLC-δ1 and –β2

Top left - The catalytic site of the rat PLC-δ1 (PDB entry: 1DJX [18]) showing the side-chains of residues involved in binding to Ins(1,4,5)P_3 and to Ca^{2+} and in the hydrolysis reaction (represented in stick). The Ca^{2+} ion is represented as a green sphere. The domain surface surrounding the catalytic site is shown in white with the hydrophobic ridge in yellow. The indicated hydrophobic residues of the ridge are those proven by substitution for Ala to give a PLC-δ1 less sensitivity to surface pressure. [13]. *Top right* – The catalytic site of the human PLC-β2 (PDB entry : 2FJU [11]). Ins(1,4,5)P_3 and Ca^{2+} are not present in the crystal. The residues of the active site, all strictly conserved between PLC-δ1 and PLC-β2, are represented. The segment of the X/Y linker resolved in the structure, occluding the active site, is represented as a meshed blue surface. *Middle* – Schematic of the reaction catalyzed by PLCs. *Bottom* - The sequence of the X/Y linkers in PLC-δ1 and PLC-β2.

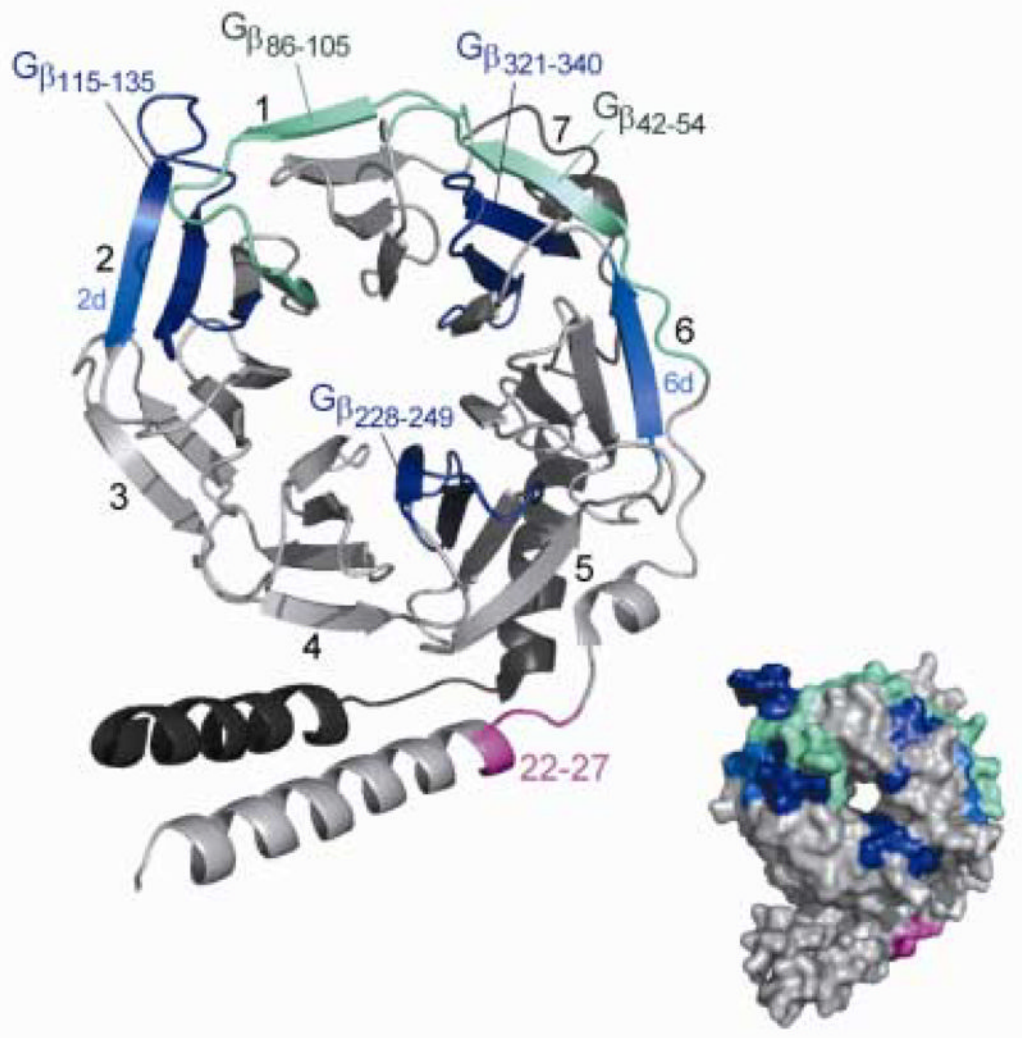

Figure 5. PLC-β2 binding sites on Gβγ

The region corresponding to the signal transfer regions [66,67] are represented in light green whereas the general PLC-β2 binding domain are in dark blue [65–67]. Additional regions found to be important for the Gβγ -activation of PLC-β2 (strands 2d and 6d) are in light blue [68]. The second-binding site for PLC-β2 found in the N-terminal region of Gβ are in magenta [71]. The Gγ subunit is colored in dark grey. The numeration of blade of the β-propeller is indicated. *Inset*: surface of the Gβγ subunit with the same color used for the representation in ribbon (PDB entry : 1OMW corresponding to Gβ1γ2)

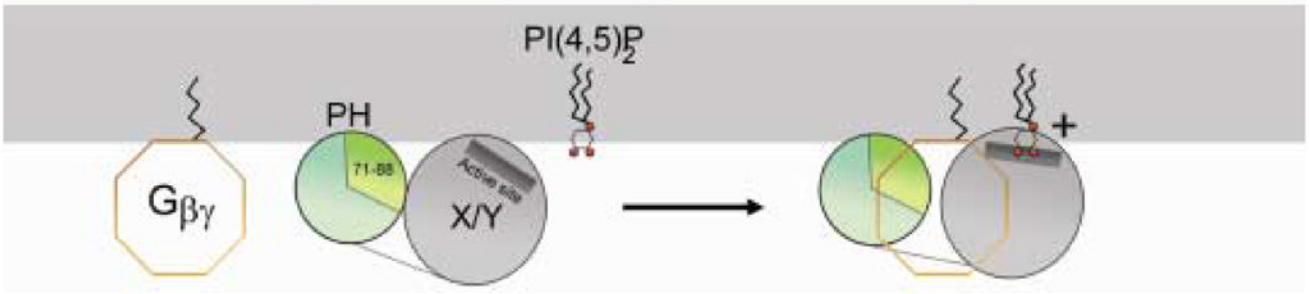

Figure 6. A model of Gβγ activation of PLC-β2

We propose that the PH domain could have an inhibitory effect on the X/Y domain through the residues 71–88 (β5/β6 loop) by maintaining the catalytic domain in a non- productive orientation. Gβγ could, by binding to the PH and X/Y domain, induce a slight conformational change allowing the X/Y domain to be correctly positioned at the membrane surface and hydrolyze the substrate.