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Review

Mammalian phosphoinositide-specific phospholipase C

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Abstract

Mammalian phosphoinositide-specific phospholipases C (PI-PLCs) are involved in most receptor-mediated signal transduction pathways. The mammalian isozymes employ a modular arrangement of domains to achieve a regulated production of two key second messengers. The roles of the PH, EF hand, C2, SH2 and SH3 modules in regulation of these enzymes and in interactions with membranes and other proteins is becoming apparent from recent structural and functional studies.

Keywords: Phospholipase C; Phosphoinositide; C2 domain; PH domain; Signal transduction

Contents

1. Introduction

Mammalian phosphoinositide-specific phospholi-

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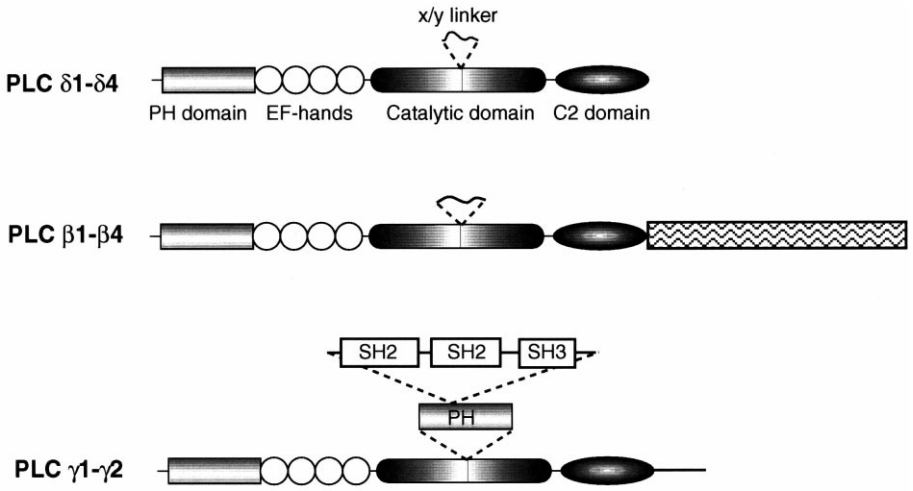


Fig. 1. A block representation of the modular domain arrangements found in the three classes of mammalian PLC isozymes.

pases C (PI-PLCs) play a key role in signal transduction by catalysing hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) to yield two second messengers, inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) and diacylglycerol. These two products of PLC catalysis mediate release of intracellular Ca^{2+} and activation of protein kinase C, respectively. The activity of PI-PLCs also control cellular concentrations of PtdIns(4,5)P_2 thereby affecting other signal transduction pathways such as that of phosphoinositide 3-kinase. Because of their substrate preference, the mammalian phospholipases are distinct from bacterial PI-PLC that cannot hydrolyse phosphorylated phosphoinositides. Another remarkable distinction from their bacterial counterparts is the diverse array of regulatory mechanisms controlling the mammalian PI-PLCs. Ten mammalian PLC isozymes have been reported: PLC-β1–4, PLC-γ1–2 and PLC-δ1–4. There is a δ-like core sequence in all of the isozymes, however, the β and γ isozymes are characterised by sequence elaboration on the δ-like core (Fig. 1). The β isozymes have C-terminal extensions of about 400 residues, and the γ isozymes have an insertion of about 500 residues between the two halves of the catalytic domain. The isozymes are also distinguished by their modes of regulation. Association with heterotrimeric G protein subunits stimulates the β-class of isozymes while the γ-class of isozymes is regulated by tyrosine kinases. Regulation of the δ-class of isozymes has not been fully characterised.

Although a three-dimensional structure has been determined only for the PLC-δ1 isozyme, this structure has served as a valuable guide for understanding the organisation of the other isozymes. One purpose of the present review is to summarise recent results concerning mammalian PI-PLCs with an emphasis on structural interpretations. The overall structure of the PLC-δ1 isozyme is illustrated in Fig. 2. The structure was determined in two parts: the isolated N-terminal PH domain [1] and the remainder of the enzyme, the catalytic core [2]. Although the catalytic core has the same activity as the intact enzyme for substrates in mixed micelles, the PH domain is essential for tethering the core to membranes and consequently enables processive catalysis of membrane-resident substrates.

2. The PH domain

The N-terminal region of PLC-δ1 contains a PH domain, a module consisting of about 120 residues that was first described in pleckstrin and subsequently reported to be present in more than 100 proteins. The structures of several PH domains have been determined and show that the domains have a remarkably conserved three-dimensional architecture despite only very limited sequence similarity. The fold of the PLC-δ1 PH domain is illustrated in Fig. 3 [1]. The domain consists of seven antiparallel β strands arranged into a barrel-like structure with one half of the barrel consisting of a three-stranded sheet, another of a four-stranded sheet and a bottom

formed by a C-terminal α-helix. Because proteins containing PH domains are capable of membrane association, it is thought that membrane binding may be a universal feature of these domains. Nevertheless, the lipid binding specificity has been clearly demonstrated for only a few PH domains. Stereospecific, high-affinity lipid head-group binding was first observed for the PLC-δ1–PH domain [3]. PLC-δ1 is associated with the plasma membrane in unstimulated cells, and the PH domain of PLC-δ1 is both necessary and sufficient for this plasma membrane localisation in vivo (Fig. 3A) [4]. Using green fluorescent protein (GFP) fusions with the PLC-δ1–PH domain, it has been shown that activation of endogenous PLCs by either ionophores or agonist-induced receptor stimulation results in transient dislocation of the PH domain fusions from the plasma membrane [5,6]. This is consistent with a model in which PLC activity decreases the concentration of $PtdIns(4,5)P_2$ and increases the concentration of the product of the reaction, IP_3, to enable feedback regulation of PLC-δ1 by transient dislocation from the plasma membrane. The structure of the PLC-δ1–PH domain in a complex with IP_3 shows that IP_3 binds

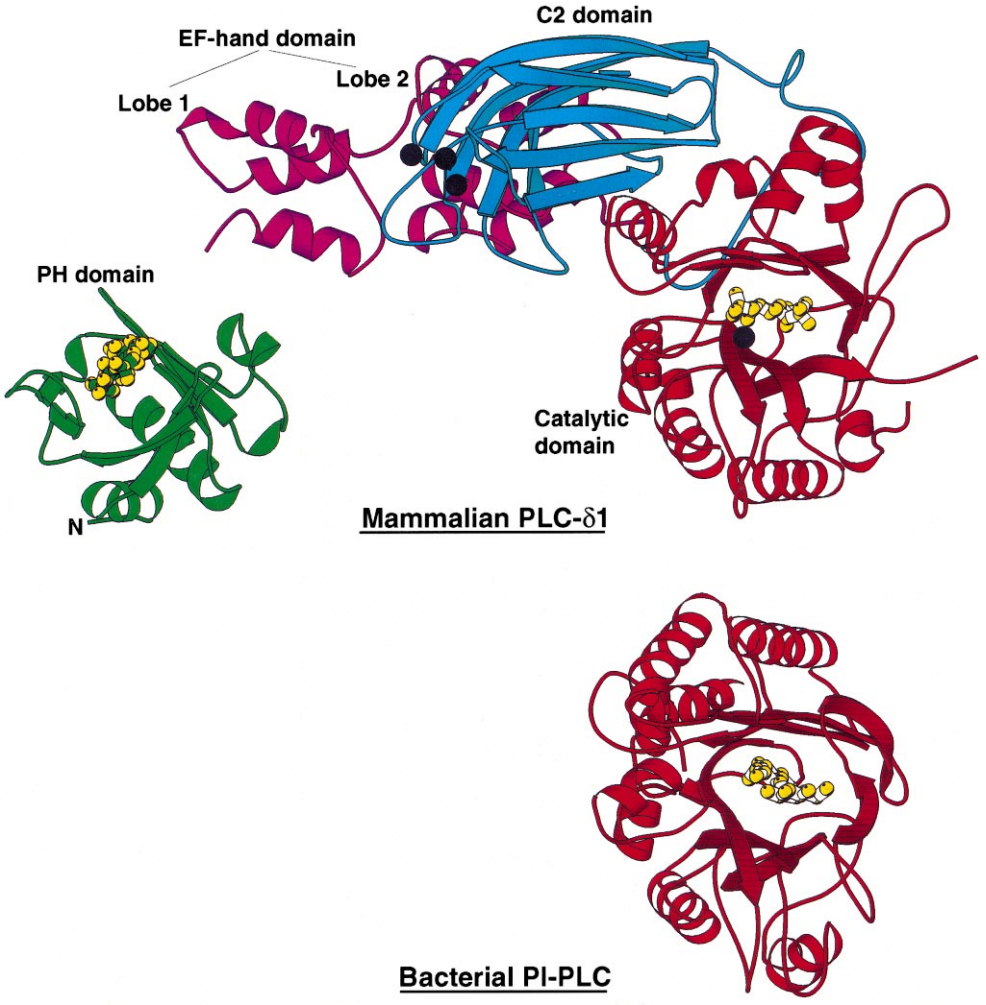


Fig. 2. An overall schematic of the PLC-δ1 from rat (upper) and the PI-PLC from *Bacillus cereus* (lower, PDB entry code 1PTD). The structure of the isolated PH domain from PLC-δ1 (pdb entry code 1MAI) was determined independently of the structure of the remainder of the enzyme (pdb entry code 2ISD). The placement of the PH domain relative to the remainder of the enzyme is arbitrary; however, the view for both parts of the enzyme is intended to suggest the view onto the enzyme from the membrane surface. The Ca^{2+} binding sites in the catalytic and C2 domains are shown as black spheres. $InsP_3$ bound in the active site and in the PH domain of PLC-δ1 is shown in yellow CPK representation. Inositol in the *B. cereus* PI-PLC is also shown in CPK.

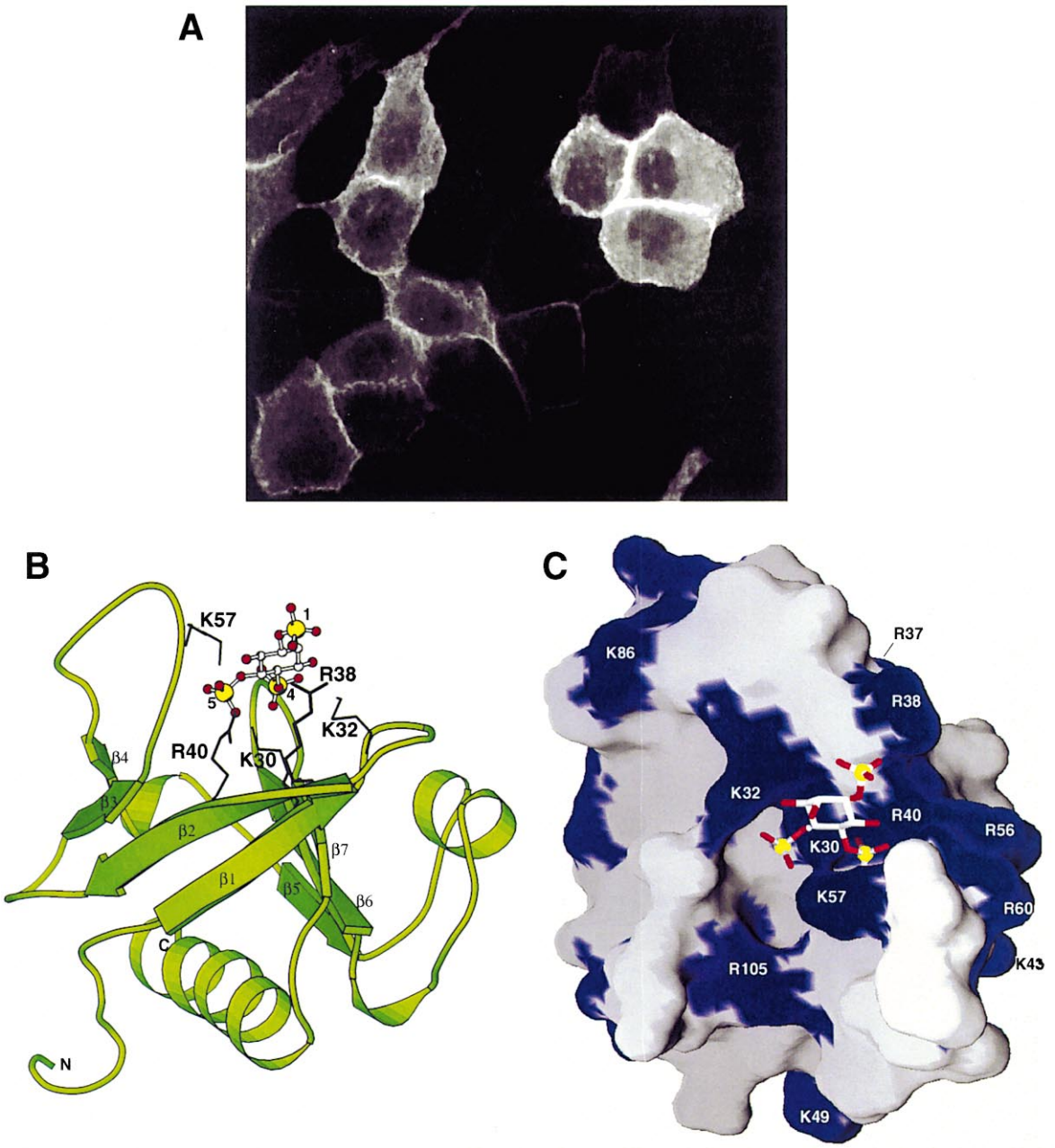


Fig. 3. The PH domain of PLC-δ1. (A) Subcellular localisation of a green fluorescent protein (GFP) fusion of PLC-δ1 PH domain expressed in MDCK cells. The PH domain, like the full-length enzyme, localises at the plasma membrane. (B) A schematic representation of the PLC-δ1 PH domain showing the basic residues whose side chains interact with $InsP_3$ in the crystal structure and have been shown by mutagenesis to be essential for $InsP_3$ binding in solution. (C) A surface representation of the PH domain with a stick model of bound $InsP_3$. The view chosen is meant to represent a view from the surface of the membrane. Lysine and arginine residues are coloured in blue.

at the positively charged end of a highly polar domain [1]. High-affinity, specific interaction between the PLC-δ1–PH and IP_3 (a K_d of 210 nM) is accomplished via an extensive network of hydrogen bonds, involving nine amino acids and primarily 4- and 5-phosphate groups of IP_3, plus a van der Waals contact between the inositol ring and W36. Most of the residues involved in these interactions are located in

strands β1 and β2 and the connecting loop β1/β2 (K30, K32, W36, R40) as well as in loop β3/β4 (E54, S55, R56 and K57) and one residue in loop β6/β7 (T107). The relevance of the interactions observed in the crystal structure is confirmed by mutagenesis which showed that all the basic residues whose side chains are hydrogen bonded to IP_3 in the crystal structure are critical for membrane binding in vitro and in vivo (Fig. 3) [7].

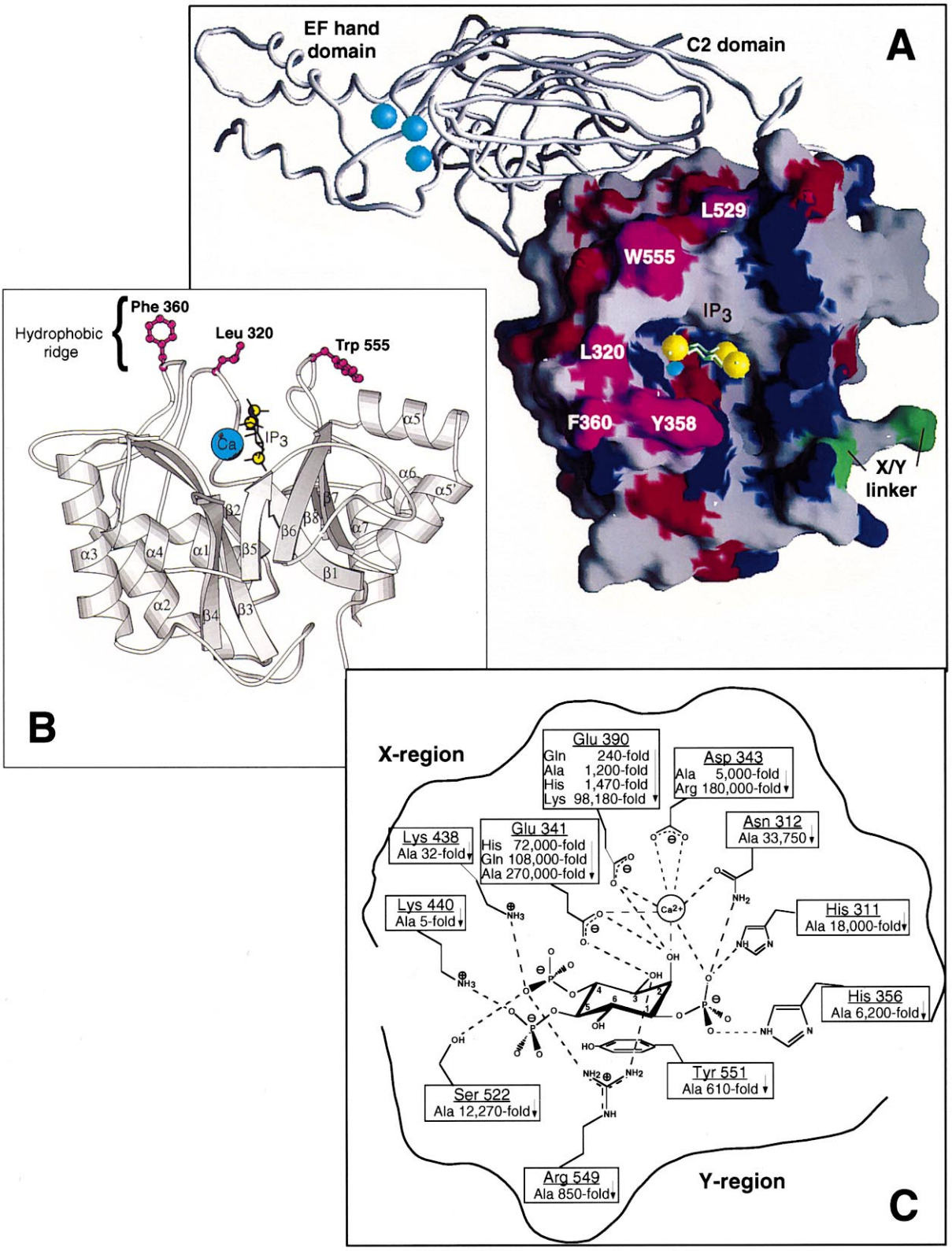
The crystal structure of the intact PLC-δ1 enzyme showed that the PH domain was disordered in the crystals, indicating that the PH domain is only flexibly attached to the catalytic core of PLC-δ1 [8]. The presence of the PH domain clearly results in enhanced catalysis by tethering the enzyme on the membrane surface where the effective substrate concentration is much greater than the bulk concentration [9,10]. In addition, recent studies suggest that despite the flexible tether to the remainder of the enzyme, PtdIns(4,5)P_2 binding to the PH domain may also directly enhance the catalytic activity of PLC-δ1 via an allosteric mechanism [11,12].

The residues interacting with IP_3 in PLC-δ1–PH are not conserved among the various mammalian PI-PLC classes. Even among the PLC-δ isozymes, these residues are not strictly conserved, suggesting that the high-affinity PtdIns(4,5)P_2 binding may be unique for the PLC-δ1–PH domain. Recent studies have begun to shed light on the functions of the PH domains in the other isozymes. The PH domain from PLC-γ1 was shown to bind PtdIns(3,4,5)P_3 with affinity sufficient to bring about translocation to membranes of green fluorescent protein fusions containing the PLC-γ1–PH domain in response to increases in PtdIns(3,4,5)P_3 arising from phosphoinositide 3-kinase activity [13]. Mutations in the loop β3/β4 that inhibited PtdIns(3,4,5)P_3 binding in vitro also abolished membrane localisation in vivo. In addition to the PH domain of PLC-γ, the SH2 domains of PLC-γ bind to PtdIns(3,4,5)P_3 with high affinity (a K_d of 2.4 μM for the second SH2 domain of PLC-γ1), thereby perhaps contributing to tighter interaction of PLC-γ with the plasma membrane [14,15]. The translocation of PLC-γ in response to increased levels of PtdIns(3,4,5)P_3 is likely to be part of a mechanism whereby PtdIns(3,4,5)P_3 activates PLC-γ.

Sequence analysis of PH domains of the mammalian PLC-β isozymes would suggest that they are not capable of making the same interactions with phosphoinositides as PLC-δ1 [16]. Using photoaffinity-labelled phosphoinositide analogues, it has been shown that the PLC-β1, -β2 and -β3 isozymes do not have a high-affinity PtdIns(4,5)P_2 or PtdIns(3,4,5)P_3 binding site [17]. However, the PH domains of PLC-β1 and -β2 isozymes bind tightly to membranes composed of a neutral lipid, phosphatidylcholine (the K_d is 28 μM for PLC-β1 and 44 μM for PLC-β2), and this binding is not affected by addition of PtdIns(4,5)P_2 or phosphatidylserine [18]. The PH domain and a sequence just following it are essential for interaction with and stimulation of PLC-β by Gβγ subunits. However, this interaction is subsequent to membrane binding by the PH domain and does not contribute to membrane recruitment of PLC-β1 and -β2 [18–20].

3. The EF-hand domain

The EF-hand domain is a region of the enzyme that forms a flexible tether to the PH domain (Fig. 2). The EF-hand domain consists of four helix–loop–helix motifs arrayed in two lobes (each containing two EF-hands) in a manner that resembles calmodulin. The first lobe of the domain appears to be much more flexible than the second lobe and is only partially visible in the crystal structures of the catalytic core [2,21]. Even in the structure of the intact PLC-δ1, which was crystallised in the same crystal form as the catalytic core (Δ(1–132) PLC-δ1), the PH domain and the first EF-hand were not visible, corroborating the intrinsic flexibility of the PH/EF-hand domains connection. Although the first two EF-hands in PLC-δ1 have residues required for calcium binding, there is no experimental evidence that the domain binds calcium. In fact, the stoichiometry of calcium binding, as determined by isothermal titration calorimetry, for the PH-domain truncated PLC-δ1 catalytic core and mutants of it, is consistent with calcium binding only to the C2 domain (three sites) and to the catalytic domain (one site), but not to the EF-hands [22]. The second lobe of the EF-hand domain makes interactions with the C-terminal domain of PLC-δ1. Deletion variants of PLC-δ1 and PLC-γ lacking this second lobe are inactive, probably due to destabilising the fold of the enzyme [23,24]. Although sequence similarity throughout the EF-


EF hand domain
C2 domain
A
L529
W555
IP3
L320
F360
Y358
X/Y linker
Hydrophobic ridge
Phe 360
Leu 320
Trp 555
Ca
IP3
α5
α6
α5'
β2
β8
β7
β6
α7
α3
α4
α1
β5
β1
α2
β4
β3
B
X-region
Glu 390
Gln 240-fold
Ala 1,200-fold
His 1,470-fold
Lys 98,180-fold
Asp 343
Ala 5,000-fold
Arg 180,000-fold
Asn 312
Ala 33,750
Lys 438
Ala 32-fold
Glu 341
His 72,000-fold
Gln 108,000-fold
Ala 270,000-fold
Lys 440
Ala 5-fold
His 311
Ala 18,000-fold
His 356
Ala 6,200-fold
Tyr 551
Ala 610-fold
Ser 522
Ala 12,270-fold
Arg 549
Ala 850-fold
Y-region
C

hand domain is very low among the PLC isozymes, examination of the predicted secondary structures in conjunction with the sequence alignments suggests that all of the mammalian isozymes are likely to have a similarly structured domain in this region. The EF-hand domain is connected to the catalytic domain via a long, well-ordered linker sequence.

4. Catalytic domain

The catalytic domain of PLC-δ1 consists of a distorted $(\beta/\alpha)_8$ barrel (Fig. 4). A similar β/α fold is also present in a bacterial PI-PLC [25]. Although the β/α barrel architecture is the most common for enzymes in general and it is likely that many such enzymes have arisen through convergent evolution, the mammalian and bacterial enzymes have in common structural features that suggest a divergence from a common ancestor (Fig. 2). There is a widening of the barrel between strands four and five that results in limited hydrogen bonding between these strands. The pattern of alternating β-strands and α-helices is also interrupted in this region with a wide loop connecting strands four and five in the bacterial enzyme and the linker insertion connecting these strands in the mammalian enzyme. In addition, both mammalian and bacterial PI-PLCs show similar placement of catalytic residues, in particular two catalytic histidines, providing another convincing evidence of an evolutionary relationship between the enzymes.

The residues that form the two halves of the catalytic barrel are separated in the sequence by a linker insertion. The sequences that form the two halves of a single three-dimensional domain are usually referred to as the X and Y regions of homology. The X region shows the greatest sequence conservation among the mammalian isozymes as well as the greatest similarity among species. The active site of PLC-δ1 is located at the C-terminal end of the β-barrel, in a rather wide, solvent-accessible cleft (Fig. 4). The affinity of the PLC-δ1 active site for PtdIns(4,5)P_2 is much lower ($K_d > 0.1$ mM) compared with the PH domain [17]. Many of the interactions that the enzyme makes with PtdIns(4,5)P_2 have been inferred from a series of structures of the PLC-δ1 in complexes with the Ca^{2+}-cofactor and inositol phosphates which mimic substrates and the reaction intermediate [26]. The interactions between the catalytic domain and the product of the reaction, IP_3, are summarised in Fig. 4C. The inositol ring fits edge-on in the active site cleft, with all groups of IP_3, except for the 6-hydroxyl group, interacting with the enzyme and the catalytic calcium ion that sits at the bottom of the active site. The preference of mammalian PI-PLCs for PtdIns(4,5)P_2 over PtdIns(4)P or PtdIns is the consequence of various salt bridges between the 4- and 5-phosphoryl groups and a cluster of basic residues (Lys 438, Lys 440, and Arg 549) in the active site.

The enzyme catalyses hydrolysis via a two-step mechanism involving a Ca^{2+} cofactor (Fig. 5). The cofactor makes a direct interaction with the 2-OH of the substrate and its role is to lower the pK_a of the 2-OH thus facilitating its deprotonation prior to nucleophilic attack on the 1-phosphate. The Ca^{2+} ion is also likely to neutralise the negative charge developed in the transition state of the reaction. The structure of the enzyme in a complex with a 1,2-cyclic phosphate intermediate analogue suggests that the Ca^{2+} also makes a direct interaction with the

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Fig. 4. The catalytic domain of PLC-δ1. (A) An overall view of the catalytic core of the enzyme with the catalytic domain shown as a molecular surface superimposed on a worm representation of the remainder of the enzyme. Basic residues are coloured blue and acidic residues coloured red. Residues of the hydrophobic ridge surrounding one side of the active-site opening are coloured purple. Residues from this ridge penetrate into lipid layers during interfacial catalysis. Coloured in green is the surface of the residues that delineate the ends of the disordered X/Y linker insertion (Fig. 1). $InsP_3$ bound in the active site is shown in ball-and-stick representation with the phosphates as large yellow spheres. The Ca^{2+} bound in the active site and the metal binding sites in the C2 domain are shown as cyan spheres. (B) A ribbon representation of the catalytic β/α domain indicating the elements of secondary structure and the residues from the hydrophobic ridge whose mutation render the activity of the enzyme less sensitive to surface pressure in monolayer assays. (C) Interactions in the active site with $InsP_3$. All of the residues interacting with $InsP_3$ in the crystal structure have been shown by mutagenesis to be important for catalytic activity of the enzyme. In the boxes associated with each of the interacting residues are listed mutations and the fold reduction they cause in hydrolysis of PtdIns(4,5)P_2 in mixed micelles with 50 μM Ca^{2+}.

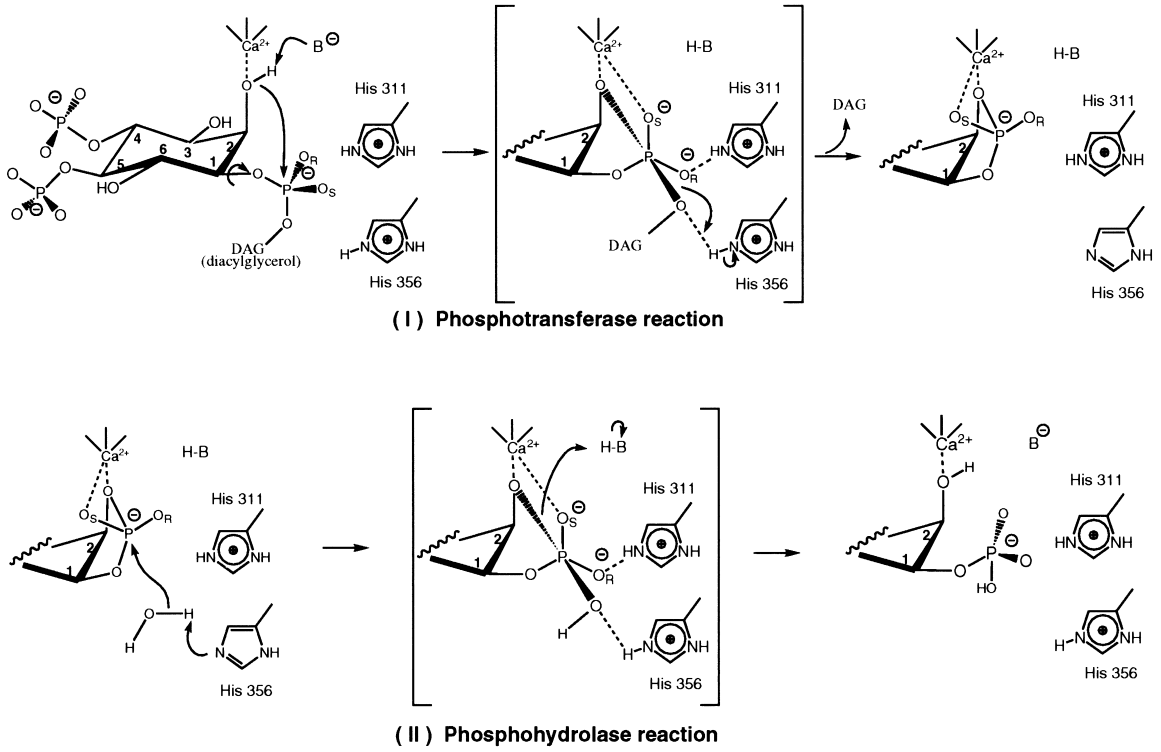

Fig. 5. A summary of the two-step catalytic mechanism of mammalian PLCs. The reaction proceeds by general acid/base catalysis. In the first step, a general base from the protein activates the 2-OH of the inositol to enable nucleophilic attack on the 1-phosphate. The identity of the general base has not been conclusively shown but is likely to be His 311. A general acid (probably His 356) protonates the DAG leaving group. The product of the first step of the reaction is a 1,2-cyclic inositol 4,5-bisphosphate. The Ca^{2+} cofactor makes an additional interaction with the inositol phosphate in the transition state (represented in square brackets) and the cyclic product of the first step. This interaction is probably critical for the relatively slow release of the cyclic inositol phosphate, and enables efficient generation of the acyclic product. In the second step, general acid/base catalysis is used to hydrolyse the cyclic intermediate yielding the acyclic product.

1-phosphate in the transition state and the intermediate of the reaction (Fig. 5). This additional interaction with the Ca^{2+}-cofactor may explain why the mammalian enzyme tends to retain the cyclic phosphate intermediate in the active site and complete the second step of the reaction to generate the acyclic product. This contrasts with the bacterial enzyme, which is Ca^{2+}-independent and for which the 1,2-cyclic inositolphosphate is the principal product of the reaction. The importance of the residues interacting with the PtdIns(4,5)P_2 head group have been verified by a systematic series of site-specific mutants of the enzyme [27]. Mutations of these residues produce substantial decreases in the rates of catalysis (up to 270 000-fold) (Fig. 4C).

Although the IP_3/PLC-δ1 complex has been helpful to understand the interaction with the head group, studies with short-chain phospholipids and glycerophospholipids indicate that there is rate acceleration for monomeric substrates that have lipid moieties [28,29]. This suggests that not all of the interactions that the enzyme makes with the substrate are with the head group. Studies of the catalysis of mammalian PLCs for phospholipid substrates in monolayers show that the enzyme activity decreases with increasing monolayer surface pressure. This has been attributed to the enzyme penetrating into the monolayer during catalysis. The structure of PLC-δ1 showed a hydrophobic ridge surrounding one end of the active site opening (Fig. 4A,B). Based on the structure of the enzyme in a complex with the detergent CHAPSO, it was proposed that this hydrophobic ridge is the portion of the enzyme that penetrates into the membrane during catalysis. Consistent with this proposal, it was found that when these exposed hydrophobic residues were mutated to ala-

nine, the surface pressure dependence of the enzymatic activity was decreased [27]. Surprisingly, these hydrophobic ridge mutants displayed activities that were about threefold greater than the wild-type enzyme. This suggests that rather than facilitating catalysis, the membrane penetration is inhibiting the enzyme. It may be that this is a mechanism that has evolved to regulate the PLC activity. The mechanism whereby the hydrophobic ridge penetration decreases activity has not been demonstrated, but restriction of substrate entry into or product exit from the active site might be possible, given that the hydrophobic ridge is positioned at the rim of the active site opening. Regulation of diffusion into and out of the active site opening may also be the basis of a very different sort of inhibitory mechanism down-regulating the basal rate of the PLC-γ isozymes.

5. The X/Y linker region

The sequence connecting the X and the Y halves of the catalytic domain is highly divergent among the mammalian PLCs. For PLC-δ1, this region is disordered in the structure and occurs between strand β4 and helix α4. The 46-residue sequence in PLC-δ1 has a large number of negatively charged residues and proteolysis in this region activates the enzyme [30]. Several regulators of PLC-δ1, such as sphingosine and spermine, require an intact X/Y linker region for their effect on the enzymatic activity in vitro [31]. Whether there may be cellular factors capable of interacting with this region that modulate the activity of PLC-δ1 in vivo is one possibility of regulation of the PLC-δ isozymes that has not been explored.

The PLC-γ isozymes have evolved regulation by the X/Y linker region to an exquisite degree. These isozymes have two SH2 domains followed by an SH3 domain in this region. The SH2/SH2/SH3 domain array is contained within a sequence that has been proposed to be a second, internal PH domain. The SH2/SH2/SH3 domain array in this model would be inserted into a loop between two β-strands (β3/β4) without disrupting the fold of the putative internal PH domain. Data suggest that this X/Y linker array has a role not only in the activation of the enzyme by tyrosine kinases, but also in the repression of basal PLC-γ activity [32].

The SH2 domains of this region are essential for activation of the enzyme by tyrosine kinases. Both binding of tyrosine-phosphorylated receptor to the SH2 domains and subsequent phosphorylation of tyrosine residues in the X/Y linker region and in the tail of PLC-γ contribute to increased enzyme activity. The structure of a complex of the C-terminal SH2 domain bound to a phosphotyrosine-containing peptide from the PDGF receptor is illustrated in Fig. 6 [33]. A positively charged pocket at one end of a groove on the surface of the domain accommodates the phosphotyrosine, while the other end of the groove that is lined with a set of hydrophobic residues interacts with the hydrophobic positions of the receptor-derived peptide. Recently, it was shown that SH2 domains of the PLC-γ isozyme can directly bind PtdIns(3,4,5)P3, and that this high-affinity binding ($K_d = 2.4$ μM) can compete with binding of tyrosine phosphorylated receptors to SH2 domains of PLC-γ [14,15]. This PtdIns(3,4,5)P_3 interaction with SH2 domains may act simultaneously with the PtdIns-(3,4,5)P_3 binding to the N-terminal PH domain, enabling membrane recruitment and/or stabilisation of PLC-γ on the membrane.

The NMR structure of the SH3 domain from PLC-γ1 (Fig. 6) shows that it has a hydrophobic cavity that is probably important for binding polyproline peptide sequences [34]. The SH3 domain of PLC-γ interacts with dynamin in vitro [35]. It is not known whether this binding occurs in vivo or is important for regulating PLC-γ1. Interactions of the SH3 domain with other proteins may have a regulatory role in vivo as has been suggested by the observation that recombinant SH3 domain from PLC-γ1 can inhibit c-kit-induced parthenogenesis in mouse eggs [36].

6. C2 domain

The C-terminal domain of PLC-δ1 consists of an eight-stranded anti-parallel β-sandwich (Fig. 7). There is a clear sequence similarity indicating that this domain is present in all of the isozymes. The domain is similar to the second conserved domain from protein kinase C, and has frequently been re-

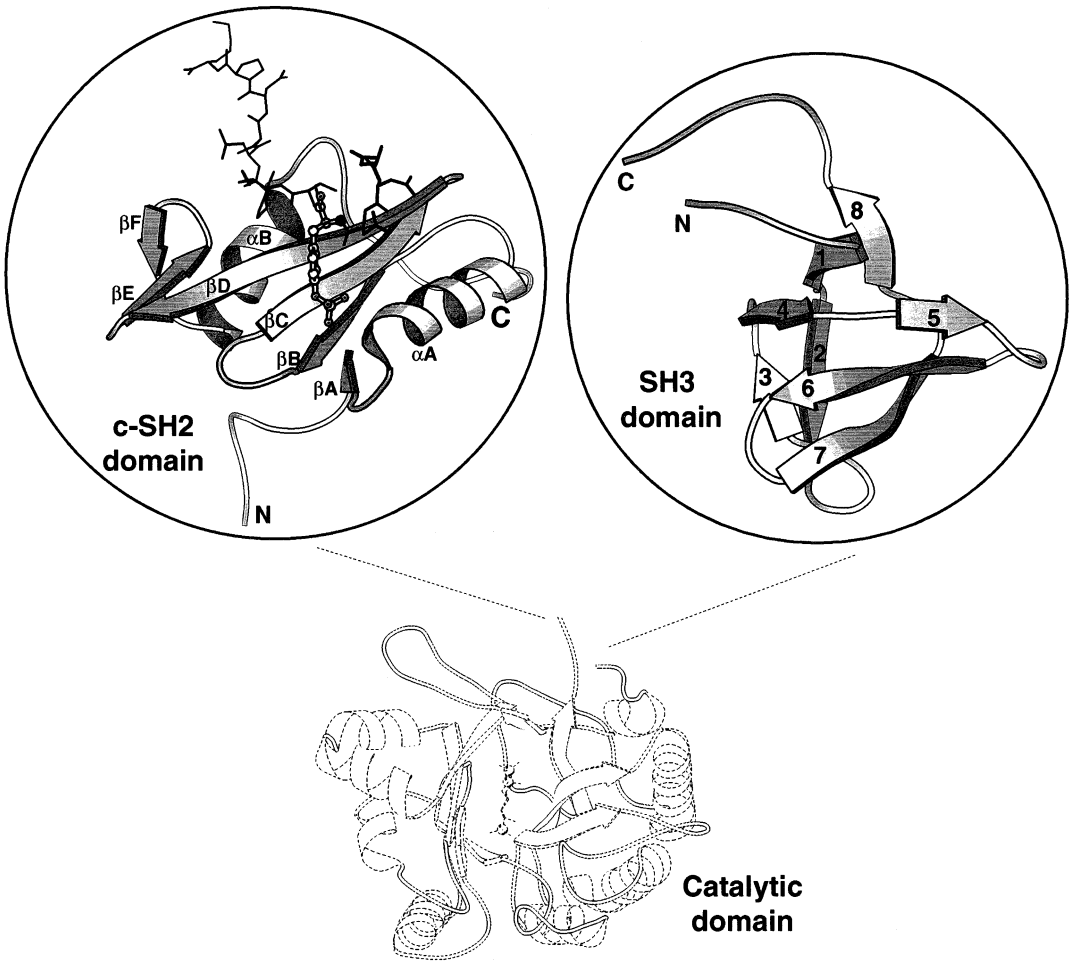

Fig. 6. A schematic representation of the structures of the C-terminal SH2 domain and the SH3 domain from PLC-γ1. The NMR structure of the C-terminal SH2 domain bound to a phosphotyrosine-containing peptide from the PDGF receptor is illustrated (pdb entry 2PLD). The phosphotyrosine is illustrated in ball-and-stick representation and the remainder of the peptide is shown as sticks. The SH3 domain structure as determined by NMR is also shown (pdb entry 1HSQ). The location on the catalytic domain into which the X/Y linker sequence containing the SH2 and SH3 domains would fit is indicated (the PLC-δ1 catalytic domain is shown dotted and not to scale).

ferred to as a 'C2' domain. The structures of several C2 domains have been determined, including those from synaptotagmin, cytosolic phospholipase A_2 (cPLA2), and protein kinases PKC-β and PKC-δ. Despite their three-dimensional similarity, the C2 domains have two different topologies that are circular permutations of each other. The topology of PLC-δ1's C2 domain is identical to that of cPLA2 and PKC-δ.

The function of C2 domains in several proteins has been the topic of intense study. Many C2 domains act as calcium-dependent lipid membrane binding modules [37]. The PLC-δ1 C2 domain crystal structure shows three metal binding sites between loops at one end of the domain (Fig. 7) and solution studies are consistent with three Ca^{2+} binding sites per domain [22]. Despite structural variation in the calcium binding regions (CBRs) among the various C2 domains, the calcium binding sites are remarkably conserved (Fig. 7). These observations suggested that the C2 domain of PLC-δ1 may also be involved in membrane binding. However, this proposal has not been supported by any binding studies. As noted previously, truncation of the N-terminal PH domain of PLC-δ1 results in an enzyme that is fully active but which binds membranes only very weakly ($K_d > 0.1$

mM) [9]. It has been shown that when the calcium-binding residues are deleted and the loops replaced by short polyglycine linkers, the enzyme activity is unaltered in vitro for the PtdIns(4,5)P_2 substrate in either micelles or vesicles. The Ca^{2+} dependence of the activity was also unaltered. These studies are consistent with cross-linking of PtdIns(4,5)P_2 that showed only PH and catalytic domain labelling. It remains to be shown whether the C2 domain might have some other role in vivo. It may be that the C2 domain of PLC-δ1 is an evolutionary remnant of an ancestral PLC that used the C2 domain for membrane binding, and that the C2 domains of mammalian PLCs have only a structural role in stabilising the enzyme.

7. The C-terminal tail

The $G\alpha_q$ family of G protein subunits activate PLC-β isozymes [38,39]. In vitro studies of purified PLC-β1, -β2 and -β3 showed only slight differences in activation with α_q, α_{11} and α_{16}. These $G\alpha_q$ subunits activate the PLC-β isozymes at much lower concentrations than Gβγ subunits. Activation by Gα subunits requires an intact C-terminal tail: C-terminal truncations of PLC-β1 and PLC-β2 in the region just beyond the C2 domain are active but not stimulated by Gα subunits [40–42]. In addition to its role in activating PLC-β in response to $G\alpha_q$, the C-terminal extension of PLC-β has a GAP activity for the G_q subunits [43]. Sequence analysis suggests that

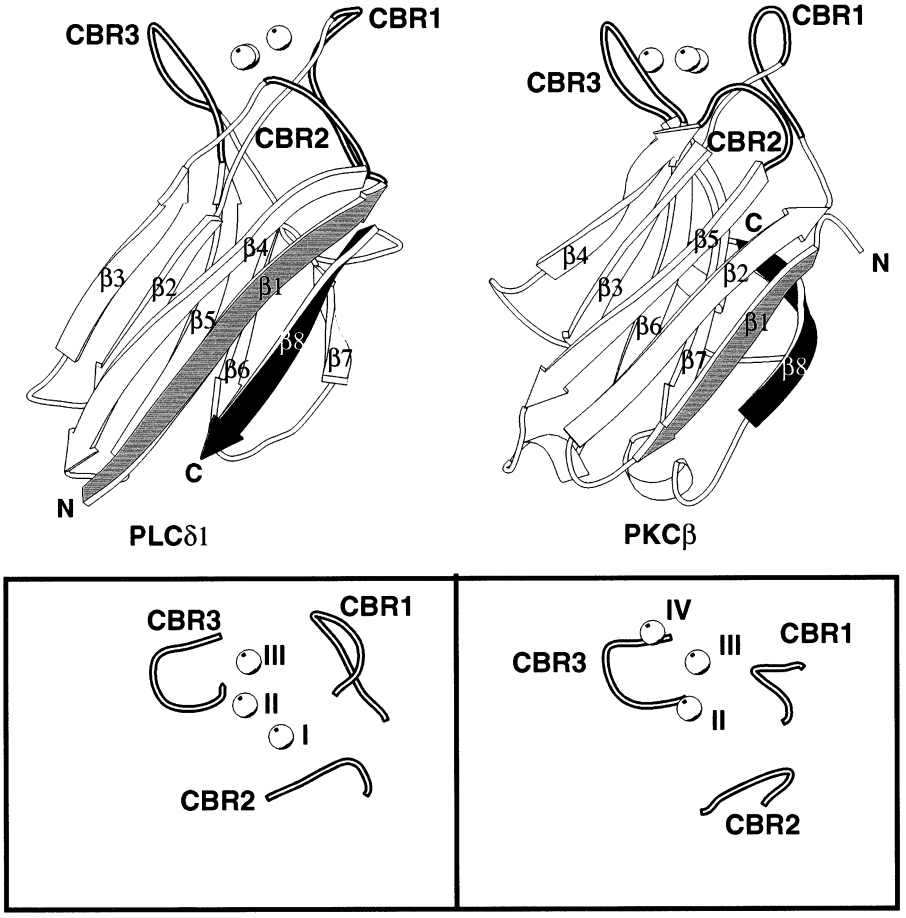

Fig. 7. The C2 domain. The C2 domains from PLC-δ1 and PKCβ (pdb entry code 1A25) are illustrated. The ribbon diagrams with shaded N- and C-terminal β-strands illustrate the overall fold and the two types of topologies that have been observed for C2 domains. These topologies are circular permutations of each other. In the lower panel, the Ca^{2+} binding regions (CBRs) are illustrated. The structure of PLC-δ1 in a complex with La^{3+} shows three metal binding sites, in agreement with the three Ca^{2+} sites demonstrated for this C2 domain in solution. The crystal structure of PKCβ's C2 domain shows three Ca^{2+} binding sites. Despite low overall sequence similarity, two of the Ca^{2+} binding sites (sites II and III) are in nearly identical positions in the two domains.

this C-terminal tail may have extensive helical content, but no structural information is available for it. Stimulation of PLC-β1 and PLC-β2 by $G\alpha_q$ subunits requires basic residues in the C-terminal tail as has been indicated by alanine substitutions [44]. Although sedimentation assays have indicated that the C-terminal tail is essential for membrane binding [45], fluorescence resonance energy transfer measurements [46] suggest that strong membrane binding can be observed even for truncation variants lacking this extension.

8. Conclusion

Although the structure of PLC-δ1 has provided a useful foundation for understanding the catalysis of all of the isozymes, regulation of PLC-δ1 in vivo remains elusive. Furthermore, understanding of the regulation of the PLC-β and γ isozymes should greatly benefit from structural studies of these isozymes and their complexes with regulatory proteins. The increasingly recognised role of translocation of PI-PLCs as a regulatory mechanism should be soon enhanced by studies of the kinetics of membrane binding and the structural determinants for this binding.

Acknowledgements

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