Journal of Biochemistry and Molecular Biology, Vol. 37, No. 6, November 2004, pp. 720-725

JOURNAL OF Biochemistry and Molecular Biology

Point Mutations in the Split PLC-γ1 PH Domain Modulate Phosphoinositide Binding

Sung-Kuk Kim[†], Sung-Mo Wee[†], Jong-Soo Chang[†,*], Taeg-Kyu Kwon[‡], Do Sik Min[§], Young Han Lee[‖] and Pann-Ghill Suh[#]

[†]Department of Life Science, College of Natural Science, Daejin University, Kyeonggido 487-711, Korea
[‡]Department of Immunology, College of Medicine, Keimyung University, Taegu 700-712, Korea
[§]Department of Molecular Biology, Pusan National University, Busan 609-735, Korea
[‖]Department of Biochemistry, College of Medicine, Yeungnam University, Taegu 705-717, Korea
[#]Department of Life Science, Pohang University of Science and Technology, Pohang 790-784, Korea

Received 25 November 2003, Accepted 16 July 2004

A number of signaling molecules contain small pleckstrin homology (PH) domains capable of binding phosphoinositides or proteins. Phospholipase C (PLC)-γ1 has two putative PH domains, an NH_2-terminal (PH_1) and a split PH domain (nPH_2 and cPH_2). We previously reported that the split PH domain of PLC-γ1 binds to phosphatidylinositol 4-phosphate (PI(4)P) and phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) (Chang *et al.*, 2002). To identify the amino acid residues responsible for binding with PI(4)P and $PI(4,5)P_2$, we used site-directed mutagenesis to replace each amino acid in the variable loop-1 (VL-1) region of the PLC-γ1 nPH_2 domain with alanine (a neutral amino acid). The phosphoinositide-binding affinity of these mutant molecules was analyzed by Dot-blot assay followed by ECL detection. We found that two PLC-γ1 nPH2 domain mutants, P500A and H503A, showed reduced affinities for phosphoinositide binding. Furthermore, these mutant PLC-γ1 molecules showed reduced $PI(4,5)P_2$ hydrolysis. Using green fluorescent protein (GFP) fusion protein system, we showed that both PH_1 and nPH_2 domains are responsible for membrane-targeted translocation of PLC-γ1 upon serum stimulation. Together, our data reveal that the amino acid residues Pro^{500} and His^{503} are critical for binding of PLC-γ1 to one of its substrates, $PI(4,5)P_2$ in the membrane.

Keywords: Dot-blotting, Phosphatidylinositol 4,5-bisphosphate, Phospholipase C-γ1, Pleckstrin homology domain, Protein-phosphoinositide interaction

*To whom correspondence should be addressed.
Tel: 82-31-539-1853; Fax: 82-31-539-1850
E-mail: jchang@daejin.ac.kr

Introduction

Many extracellular signals activate PLC-γ1, leading to production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). These secondary messengers trigger cellular signaling cascades through release of Ca^{2+} from intracellular stores and activation of protein kinase C (PKC), respectively (Berridge, 1993; Nishizuka, 1995). It was reported that overexpression of PLC-γ1 induced malignant transformation in nude mice (Chang *et al.*, 1997) and targeted deletion of PLC-γ1 resulted in embryonic lethality in mice (Ji *et al.*, 1997). Thus, it is important to elucidate the functional roles of PLC-γ1 in cell growth and differentiation.

PLC-γ1 enzyme activity is regulated through its interactions with ligand receptors and cytoplasmic proteins via its Src homology (SH) and pleckstrin homology (PH) domains (Rhee, 2001). The PH domain is a 120 amino acid region found in a large number of proteins, from yeast to mammals (Haslam *et al.*, 1993; Mayer *et al.*, 1993; Gibson *et al.*, 1994). PH domains bind with high specificity and affinity to phosphoinositides, including PI(3)P, PI(4)P, $PI(4,5)P_2$, $PI(3,4,5)P_3$ and IP_3 (Rebecchi and Scarlata, 1998; Lemmon and Ferguson, 1998; Lemmon and Ferguson, 2000). The PH domains of signaling molecules are involved in targeted translocation of the molecules to cell membranes (Lemmon *et al.*, 1996; Razzini *et al.*, 2000) and mediation of protein-protein and protein-lipid interactions including those with the βγ-subunit of heteromeric G-protein (Touhara *et al.*, 1994; Pitcher *et al.*, 1995), PKC (Yao *et al.*, 1997), actin (Yao *et al.*, 1999) and PLC-γ1 (Chang *et al.*, 2002).

PLC-γ1 has two putative PH domains: one located in the 150 N-terminal amino acids and the other split by the SH2-SH2-SH3 domains (Fig. 1). Here, we investigated the binding of the split PLC-γ1 PH domain to phosphoinositide using site-

directed mutagenesis and Dot-blot hybridization with a GST-PH fusion protein. We found that two point mutations within the PLC-γ1 split PH domain resulted in reduction of phosphoinositide binding and enzyme activity.

Materials and Methods

Reagents Anti-FLAG M5 monoclonal and anti-PLC-γ1 polyclonal antibodies were obtained from Sigma-Aldrich (St. Louis, USA). Fluorescein-conjugated Affinipure goat anti-rabbit IgG and Rhodamine-conjugated Affinipure goat anti-mouse IgG were from Jackson ImmunoResearch Laboratories (West Grove, USA), respectively. Anti-GST and horseradish peroxidase (HRP)-conjugated donkey anti-goat antibodies were from Santa Cruz Biotechnology (Santa Cruz, USA). All phospholipids, including phosphatidylethanolamine (PE), PI(4)P and PI(4,5)P_2, were from Sigma-Aldrich. PIP strips (Cat. No. P6001) were purchased from Echelon Bioscience Inc. (Salt Lake City, USA), and [^{3}H]-PIP_2 was from Perkin Elmer Life Sciences (Boston, USA).

DNA constructs The GST-PLC-γ1 PH domain fusion protein expression vectors were engineered by polymerase chain reaction (PCR) amplification of rat PLC-γ1 cDNA (Suh *et al.*, 1988). Briefly, the PH domains were PCR amplified using primers designed to introduce a 5' *Eco*RI site or a 3' *Xho*I site. The resultant PCR products were ligated into the pGEX-5X-1 vector (Amersham Pharmacia Biotech, Piscataway, USA). PCR-based site-directed mutagenesis was used to generate the appropriate point mutants. The fusion proteins were expressed in *E.coli* and purified with glutathione-sepharose 4B (Amersham Pharmacia Biotech, Richmond, USA) according to the manufacturers specifications. The GST fusion proteins used in this study were as follows: GST-PH_1 (encoding residues 25-145 of PLC-γ1), GST-nPH_2 (residues 477-547 of PLC-γ1) and GST-cPH_2 (residues 850-979 of PLC-γ1).

For mammalian expression, cDNAs encoding wild type and mutant (P500A and H503A) PLC-γ1 were ligated into the *Hind*III/*Xba*I restriction sites of the N-terminal FLAG epitope tagging vector, pFLAG-CMV-2 (Sigma-Aldrich, St. Louis, USA). Additionally, the wild type and mutant PH domains of PLC-γ1 were subcloned into the *EcoRI*/*Xba*I sites of pEGFP-C2 (Clontech, Palo Alto, USA) for generation of green fluorescent protein (GFP) fusion proteins for use in surveying the localization of the PH domains in COS-7 cells. All DNA constructs were confirmed by direct sequencing using the dideoxy-chain termination method.

Expression and purification of PLC-γ1 COS-7 cells grown in Dulbecco's modified Eagle's medium (DMEM) on 100-mm culture dishes were transfected with 5 μg of plasmids encoding FLAG-tagged wild type or mutant enzymes and 10 μl of Lipofectamine (Gibco/BRL, Rockville, USA), according to the manufacturer's specifications. Two days after transfection, cells were harvested, and the native and mutant PLC-γ1 proteins were purified with a pFLAG-CMV-2 purification kit (Sigma-Aldrich, St. Louis, USA).

Immunofluorescent microscopy COS-7 cells were seeded on coverslips in 6-well plates, transfected with 5 μl of Lipofectamine and 2 μg of pEGFP-PH domain fusion vector or vector alone (control), and then incubated for 2 days in DMEM with 0.5% fetal bovine serum (FBS). Cells were then stimulated with 20% fresh serum for 20 min (or left unstimulated as a control), fixed with 4% paraformaldehyde in PBS for 10 min at 37°C, and GFP fusion proteins were visualized using a fluorescent microscope (Nikon).

PLC-γ1 activity assay PLC-γ1 activity was measured as previously described (Chang *et al.*, 2002). Briefly, the substrate was prepared as sonicated vesicles of 75 mM cold PIP_2, 75 mM [^{3}H]-PIP_2 (9,000-10,000 cpm/assay, Perkin Elmer Life Sciences) and 750 mM PE in 20 mM HEPES buffer (pH 7.2) containing 30 mM KCl and 2 mM $CaCl_2$. Native or mutant PLC-γ1 (50 ng) was added to 50 μl of vesicle solution and incubated for 20 min at 30°C. Reactions were terminated by addition of 1 ml of chloroform/methanol/HCl (50 : 50 : 0.3) and 0.45 ml of 1 N HCl, vortexed and centrifuged for 10 min at 2,000 rpm. The aqueous phase (250 μl), containing [^{3}H]-IP_3, was collected and the radioactivity was measured by scintillation counter.

Dot-blot analysis The ability of the PH domains to bind different phospholipids was examined by Dot-blot analysis (Stevenson *et al.*, 1998). Briefly, chloroform-solubilized phosphoinositides (5 μg each) were spotted onto a nitrocellulose membrane (PROTRAN, Schleicher & Schuell) and dried at room temperature for 1 hr. Membranes were blocked with 2% non-fat skim milk in Tris-buffered Tween-20 (TBT) buffer for 1 hr, or Echelon strips were blocked in TBT buffer alone for 1 hr. The membranes were then incubated with purified GST, GST-PH_1, GST-nPH_2 or GST-nPH_2 (0.5 μg/ml), respectively, in blocking buffer for 14 hr at 4°C. After washes with TBT buffer, the membranes were incubated with anti-GST antibody for 2 hr at room temperature, followed by extensive washing in TBT buffer. The bound proteins were then visualized by successive incubation with HRP-conjugated anti-GST and anti-goat secondary antibodies followed by ECL detection.

Results

The PH domains of PLC-γ1 bind to phosphoinositide The PLC-γ1 molecule has two PH domains that may bind to phosphoinositide (Fig. 1). To investigate the interactions of the PLC-γ1 PH domains with phosphoinositides, we generated three GST-PLC-γ1-PH domain fusion proteins: GST-PH_1, GST-nPH_2 and GST-cPH_2. As shown in Fig. 2, we performed Dot-blot hybridization using Echelon strips and probed the strips with the GST, GST-PH_1, GST-nPH_2 and GST-cPH_2 fusion proteins followed by detection with an anti-GST antibody (Fig. 2). All the fusion proteins (except GST alone) directly bound to the various phosphatidylinositol phosphates with different affinities. PI(3)P was the preferred substrate for all tested fusion proteins, with GST-nPH_2 showing the highest affinity overall.

Membrane targeted translocation of the PLC-γ1 PH_1 and nPH_2 domains Previous studies demonstrated that the PH_1 domain of PLC-γ1 translocates from the cytosol to the plasma

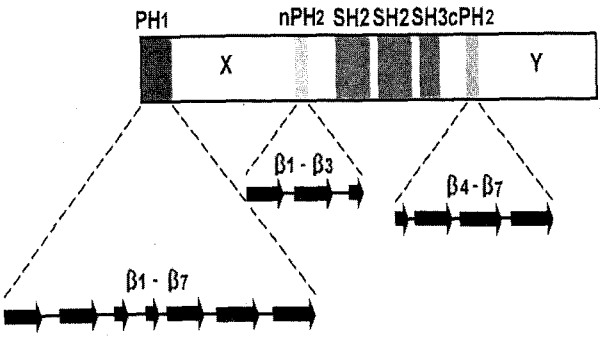

Fig. 1. PLC-γ1 has two PH domains. PLC-γ1 has two putative PH domains (PH_1 and split PH_2) in addition to its SH2n, SH2c, SH3, and catalytic X and Y domains. The split PH domain consists of the NH_2-terminal (nPH_2) and COOH-terminal (cPH_2) portions. The PH_1 domain contains 7 β-strands, and the nPH_2 and cPH_2 domains contain 3 and 4 β-strands, respectively, for protein-protein or protein-phosphoinositide interactions.

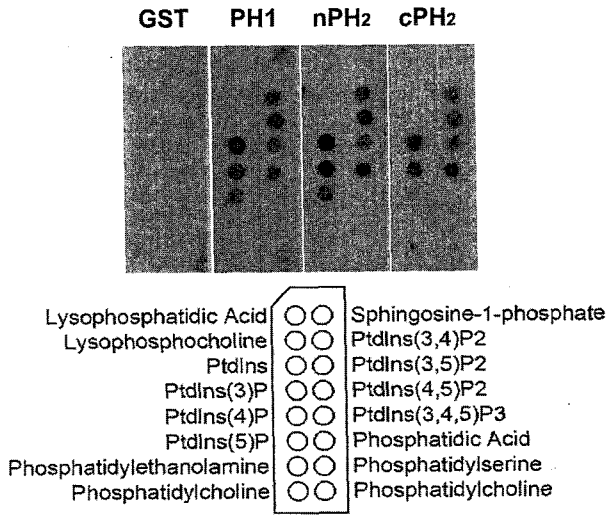

Fig. 2. Phosphoinositide-binding affinity of the PLC-γ1 PH domains. GST-PH_1, GST-nPH_2 and GST-cPH_2 fusion proteins and GST alone were overlain on Echelon filters spotted with different phosphoinositides (listed at bottom of panel). Following the extensive washing, anti-GST and HRP-conjugated donkey anti-goat antibodies were used to probe the filters, which were then visualized by ECL.

membrane following cellular stimulation with platelet derived growth factor (PDGF) or serum (Falasca *et al.*, 1998). Accordingly we used the GFP fusion proteins to investigate whether the PLC-γ1 nPH_2 domain as well as PH_1 domain localized to the membrane following serum stimulation. GFP-PH_1 and GFP-nPH_2 fusion proteins were transiently overexpressed in COS-7 cells, and the localization of fluorescent intensity was examined by fluorescent microscopy. Both proteins localized in the plasma membrane after serum stimulation for 20 min (Fig. 3c and d), whereas both were cytosolic in unstimulated cells (Fig. 3a and b). These results indicate that the PH_1 and nPH_2 domains of PLC-

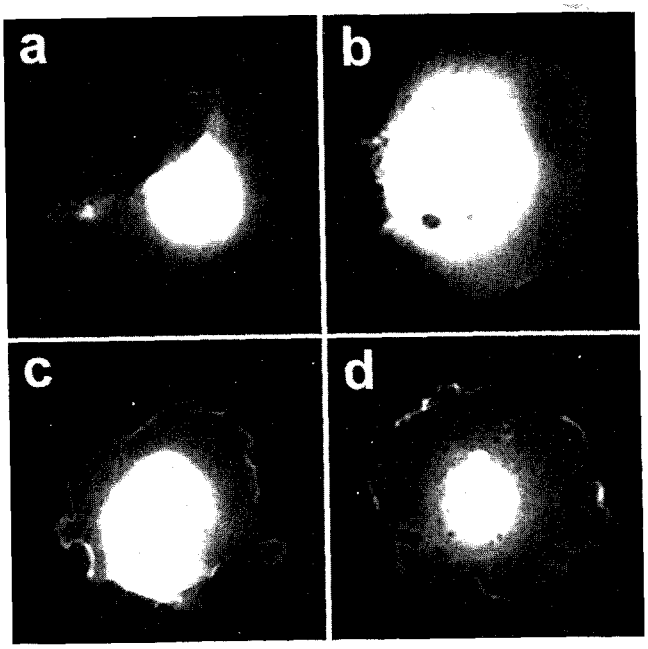

Fig. 3. Fluorescent microscopy of COS-7 cells expressing the PH domains of PLC-γ1 fused to GFP. Serum-starved cells showed a diffuse, cytoplasmic green fluorescence when expressing GFP-PH_1 (a) or GFP-nPH_2 (b). Following 20% serum stimulation for 20 min, a distinct green fluorescence is seen in the plasma membranes of cells expressing GFP-PH_1 (c) and GFP-nPH_2 (d).

γ1 both translocate from the cytosol to the plasma membrane upon agonist stimulation. Although we were unable to directly examine the localization of GFP-cPH_2 in this experiment, we suggest that it most likely localizes in the membrane following agonist stimulation, as our Dot-blotting identified direct binding of this domain to various phosphoinositides (Fig. 2).

Site-directed mutagenesis of the PH domains Since our previous data showed that the nPH_2 domain easily binds to cellular proteins on SDS-PAGE (Chang *et al.*, 2002) and the nPH_2 domain showed highest affinity for phosphoinositides (Fig. 2), we next performed site-directed mutagenesis of the nPH_2 domain in an effort to better understand the importance of each amino acid within this sequence. Our previous study (Chang *et al.*, 2002) identified 42 residues of the nPH_2 domain that are responsible for specific binding of proteins and phosphoinositides. From these 42 residues, we chose 16 for site-directed mutagenesis to alanine (a small, neutral amino acid) (Fig. 4a). The altered residues were located within the VL-1 region, which is known to be associated with phosphoinositide binding (Lemmon and Ferguson, 2000). Successful site directed mutagenesis was confirmed by direct sequencing.

Pro^{500} and His^{503} are critical for phosphoinositide binding Dot-blot analysis of the various mutant GST-nPH_2 fusion

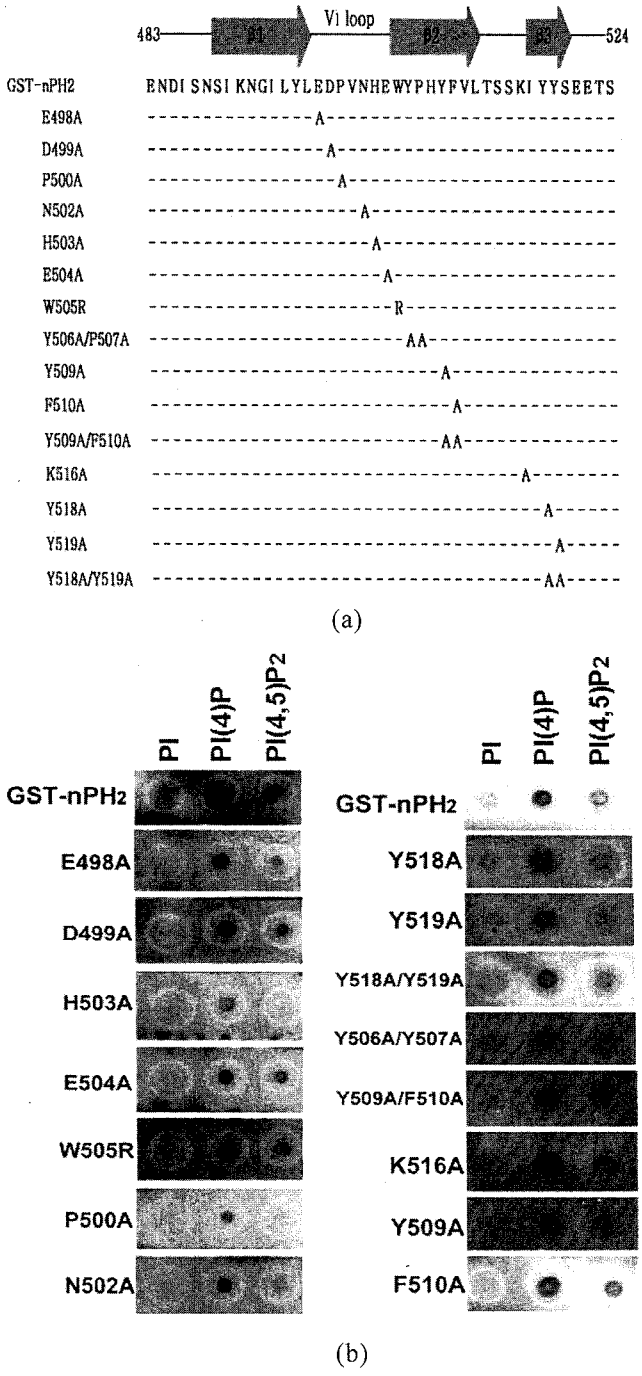

Fig. 4. Comparison of phosphoinositide binding affinities between native and mutant PLC-γ1 nPH_2 domains. (a) Amino acid sequences of the nPH_2 domain (corresponding to β-sheets 1-3) of PLC-γ1 are depicted using the standard single-letter abbreviations. The mutated amino acids are shown under the relevant wild type amino acids. (b) Mutant forms of GST-nPH_2 domain fusion proteins were used to probe nitrocellulose filters spotted with various phosphoinositides.

proteins demonstrated that two amino acid residues, Pro^{500} and His^{503}, are important for phosphoinositide binding. As shown in Fig. 4b, substitutions of alanine for Pro^{500} and His^{503} (P500A and H503A) resulted in substantially reduced affinities for phosphoinositide, in comparison to the dot intensity achieved with the native nPH_2 fusion protein.

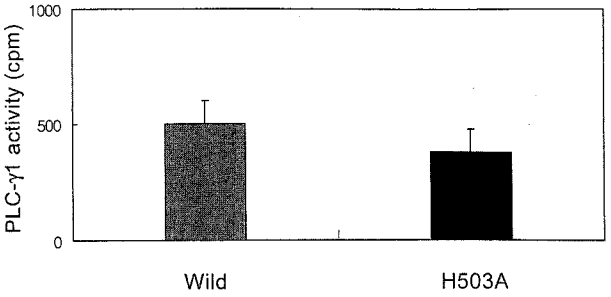

Fig. 5. PI(4,5)P_2 hydrolysis by native and H503A mutant PLC-γ1. PLC-γ1 activity was measured as described under "Experimental Procedures." The data represent the averages of duplicate determinations (mean ± range) from one of two experiments with similar results.

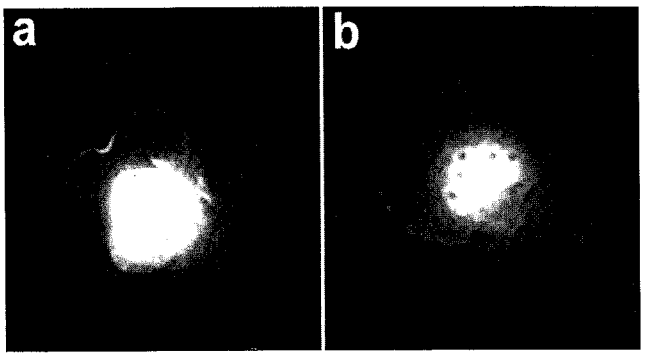

Fig. 6. Subcellular localizations of GFP-nPH_2 and GFP-H503A. COS-7 cells expressing GFP-nPH_2 (a) and GFP-H503A (b) were fixed with 4% paraformaldehyde followed by immunofluorescent microscopy. Both proteins are observed in the plasma membrane following serum stimulation.

Substrate hydrolyzing activity of H503A Next, we investigated whether the H503A point mutant showed an altered enzyme activity; we examined only the H503A mutant because our Dot-blot results indicated that the phosphoinositide binding affinities of P500A and H503A were similar. As it was previously shown that overexpression of truncated PH_1 (Falasca *et al.*, 1998) and nPH_2 (Chang *et al.*, 1996) domains in mammalian cells resulted in greatly reduced IP_3 production following PDGF treatment *in vivo*, we examined *in vitro* activity assays of affinity-purified native and mutant PLC-γ1 (H503A) with [^{3}H]-PIP_2 as a substrate, and found that the enzyme activity of the H503A mutant was reduced to 80% that of native PLC-γ1 (Fig. 5).

Finally, we examined the subcellular localization of the GFP-nPH_2-H503A mutant protein. As shown in Fig. 6, GFP-H503A was translocated to the membrane in the presence of 20% serum, as was the native protein. Thus, although the H503A mutant showed reduced affinity for PI(4,5)P_2 binding,

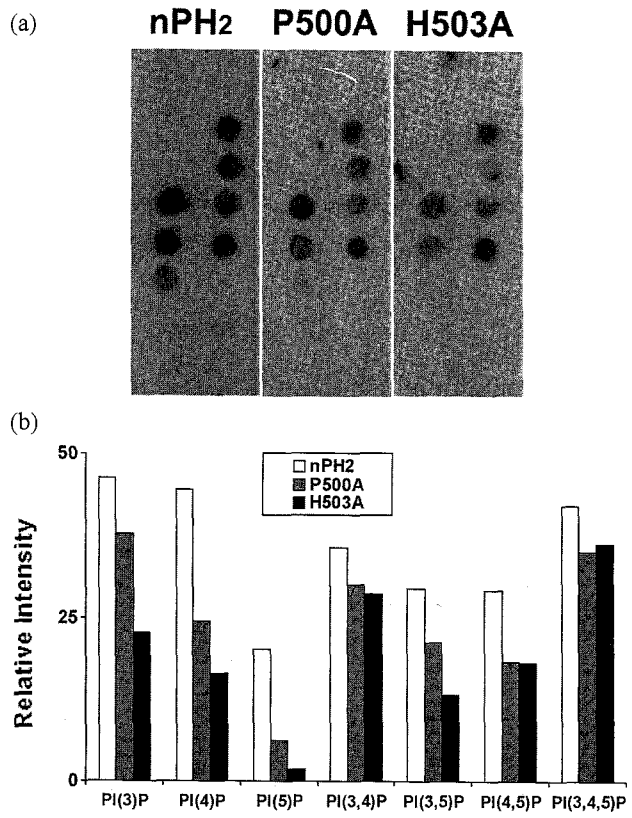

Fig. 7. Effects of point mutations within the nPH_2 domain on phosphoinositide binding. Purified GST-nPH2, GST-P500A and GST-H503A fusion proteins were overlain on Echelon strips followed by anti-GST antibody detection (a). The images of each dot were analyzed for relative intensity using the Quantity One software (Bio-Rad) (b).

its localization indicates that it might still retain binding affinity for membrane-bound phosphoinositides such as $PI(3,4,5)P_3$. To examine this possibility, we compared the binding affinities of nPH_2 and H503A fusion proteins for various phosphoinositides. As we expected, the nPH_2 and H503A fusion proteins showed similar affinities for $PI(3,4,5)P_3$ (Fig. 7a and b). However, we noted a remarkable reduction in the phosphoinositide binding affinity of H503A for PI(3)P, PI(4)P, PI(5)P and $PI(4,5)P_2$ when we compared to that of nPH_2, perhaps explaining why the GFP-H503A fusion protein showed the membrane localization upon serum stimulation with a reduced $PI(4,5)P_2$ hydrolyzing activity.

Discussion

Here, we functionally characterized the PH domain of PLC-γ1. Our results demonstrate that two single amino acid substitutions within the PH domain of PLC-γ1 can affect its phosphoinositide binding and enzyme activity. We previously reported that the PLC-γ1 nPH_2 domain binds to both phosphoinositide and the translational elongation factor-1α (EF-1α) protein (Chang *et al.*, 2002). Although EF-1α does not directly bind phosphoinositide, it accelerates hydrolysis of $PI(4,5)P_2$ by PLC-γ1. Point mutations in the nPH_2 domain (Y509A, F510A and Y509A/F510A) completely abolished the binding affinity to EF-1α (Chang *et al.*, 2002). As a complement to the previous study, we herein sought to identify amino acid residues within nPH_2 responsible for phosphoinositide binding.

Overexpressed truncated molecules of the PLC-γ1 PH_1 and split nPH_2 domains have been shown to act as dominant-negative inhibitors of PLC-γ1 activity (Chang *et al.*, 1996; Falasca *et al.*, 1998), perhaps by competitively sequestering phosphoinositides away from native PLC-γ1. Moreover, independently expressed PLC-γ1 catalytic domains (X, residues 1-516; and Y, residues 902-1290) lacking the SH2-SH2-SH3 domains showed 20-fold more $PI(4,5)P_2$ hydrolysis activity *in vitro* than did the native protein (Horstman *et al.*, 1996). Another report showed that PLC-γ1 lacking the PH_1 domain (amino acid residues 1-220) was inactive *in vitro* (Emori *et al.*, 1989). Many reports have suggested that the PLC-γ1 X and Y domains are sufficient for enzymatic activity, but it should be noted that all of these constructs contained the PH_1 and nPH_2 domains. Thus, our results and the previous works suggest that the PH domains of PLC-γ1 are essential for its membrane localization and enzyme activity.

We found that the PH_1 and nPH_2 domains both bind to phosphoinositide and both translocate from the cytosol to the plasma membrane upon serum stimulation in COS-7 cells (Fig. 3). When cells were stimulated with 200 ng/ml EGF instead of serum, we also observed membrane-targeted translocation of both PH domains (data not shown). These results indicate that agonist stimulation with serum, EGF or PDGF results in membrane-targeted translocation of PLC-γ1, and means that the PH_1 and nPH_2 domains works independently for PLC-γ1 activation in the membrane. The P500A and H503A mutants showed equally reduced intensities in our Dot-blot assay, as compared to that of the wild type nPH_2 domain. The VL-1 region (containing both P^{500} and H^{503}) has been associated with binding of negatively charged phosphoinositides (Lemmon and Ferguson, 2000); since His^{503} is a basic, positively charged residue, its replacement with a neutral residue (Ala^{503}) might have led to reduced binding of negatively charged phosphoinositides. Alternatively, we can speculate that substitution of either Pro^{500} or His^{503} with Ala may result in conformational changes within the nPH_2 domain of PLC-γ1. The H503A mutant protein still translocated to the membrane following serum treatment, perhaps indicating that some membrane-anchored phospholipid molecules, including $PI(3,4)P_2$ and $PI(3,4,5)P_3$, retained a high affinity for the mutant protein (Fig. 7).

In summary, we have shown that two point mutations within the PLC-γ1 nPH_2 domain led to reduced $PI(4,5)P_2$ hydrolysis, indicating that the PHs domains of PLC-γ1 are essential for its enzymatic activity. Our present data are the first report of cell membrane translocation of the nPH_2 domain

following agonist stimulation, and evidence that point mutations within nPH_2 domain can disrupt PLC-γ1 enzyme activity.

Acknowledgments This work was supported by a grant from the Korea Research Foundation (KRF-2002-015-CP0326).

References

Berridge, M. J. (1993) Inositol trisphosphate and calcium signaling. *Nature* **361**, 315-325.

Chang, J.-S., Min, D. S., Bae, S.-S., Kim, J. H., Lee, Y. H., Ryu, S. H. and Suh, P.-G. (1996) Overexpression of SH2-SH2-SH3 domain of phospholipase C-γ1 blocks PDGF-induced inositol phosphate generation in NIH 3T3 cells. *Mol. Cells* **6**, 259-265.

Chang, J.-S., Noh, D. Y., Park, I. A., Kim, M. J., Song, H., Ryu, S. H., and Suh, P.-G. (1997) Overexpression of phospholipase C-γ1 in rat 3Y1 fibroblast cells leads to malignant transformation. *Cancer Res.* **57**, 5465-5468.

Chang, J.-S., Seok, H., Kwon, T.-K., Min, D. S., Ahn, B.-H., Lee, Y. H., Suh, J.-W., Kim, J.-W., Iwashita, S., Omori, A., Ichinose, S., Numata, O., Seo, J.-K., Oh, Y.-S. and Suh, P.-G. (2002) Interaction of Elongation factor-1α and pleckstrin homology domain of phospholipase C-γ1 with activating its activity. *J. Biol. Chem.* **277**, 19697-19702.

Emori, Y., Homma, Y., Sorimachi, H., Kawasaki, H., Nakanishi, O., Suzuki, K. and Takenawa, T. (1989) A second type of rat phosphoinositide-specific phospholipase C containing a src-related sequence not essential for phosphoinositide-hydrolyzing activity. *J. Biol. Chem.* **264**, 21885-21890.

Falasca, M., Logan, S. K., Lehto, V. P., Baccante, G., Lemmon, M. A. and Schlessinger, J. (1998) Activation of phospholipase Cγ by PI 3-kinase-induced PH domain-mediated membrane targeting. *EMBO J.* **17**, 414-422.

Gibson, T. J., Hyvonen, M., Musacchio, A., Saraste, M. and Birney, E. (1994) PH domain: the first anniversary. *Trends Biochem. Sci.* **19**, 349-353.

Haslam, R. J., Koide, H. B. and Hemmings, B. A. (1993) Pleckstrin domain homology. *Nature* **363**, 309-310.

Horstman, D. A., DeStefano, K. and Carpenter, G. (1996) Enhanced phospholipase C-γ1 activity produced by association of independently expressed X and Y domain polypeptides. *Proc. Natl. Acad. Sci. USA* **93**, 7518-7521.

Ji, Q.-S., Winnier, G. E., Niswender, K. D., Horstman, D., Wisdom, R., Magnuson, M. A. and Carpenter, G. (1997) Essential role of the tyrosine kinase substrate phospholipase C-γ1 in mammalian growth and development. *Proc. Natl. Acad. Sci. USA* **94**, 2999-3003.

Lemmon, M. A., Ferguson, K. M. and Schlessinger, J. (1996) PH domains: diverse sequences with a common fold recruit signaling molecules to the cell surface. *Cell* **85**, 621-624.

Lemmon, M. A. and Ferguson, K. M. (1998) Pleckstrin homology domains. *Curr. Top. Microbiol.* **228**, 39-74.

Lemmon, M. A. and Ferguson, K. M. (2000) Signal-dependent membrane targeting by pleckstrin homology (PH) domains. *Biochemical J.* **350**, 1-18.

Mayer, B. J., Ren, R., Clark, K. L. and Baltimore, D. (1993) A putative modular domain in diverse signaling proteins. *Cell* **73**, 629-630.

Nishizuka, Y. (1995) Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* **9**, 484-496.

Pitcher, J. A., Touhara, K., Payne, E. S. and Lefkovitz, R. J. (1995) Pleckstrin homology domain-mediated membrane association and activation of the β-adrenergic receptor kinase requires coordinate interaction with Gβγ subunits and lipid. *J. Biol. Chem.* **270**, 11707-11710.

Razzini, G., Brancaccio, A., Lemmon, M. A., Guarnier, S. and Falasca, M. (2000) The role of the pleckstrin homology domain in membrane targeting and activation of phospholipase C-β1. *J. Biol. Chem.* **275**, 14873-14881.

Rebecchi, M. J. and Scarlata, S. (1998) Pleckstrin homology domains: a common fold with diverse functions. *Annu. Rev. Biophys. Biomol. Struct.* **27**, 503-528.

Rhee, S. G. (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu. Rev. Biochem.* **70**, 281-312.

Stevenson, J. M., Perera, I. Y. and Boss, W. F. (1998) A phosphatidylinositol 4-kinase pleckstrin homology domain that binds to phosphatidylinositol 4-monophosphate. *J. Biol. Chem.* **273**, 22761-22767.

Suh, P.-G., Ryu, S. H., Moon, K. H., Suh, H. W. and Rhee, S. G. (1988) Inositol phospholipids-specific phospholipase C: complete cDNA and protein sequences and sequence homology to tyrosine kinase-related oncogene products. *Proc. Natl. Acad. Sci. USA* **85**, 5419-5423.

Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G. and Lefkowitz, R. J. (1994) Binding of G protein βγ subunit to pleckstrin homology domains. *J. Biol. Chem.* **269**, 10217-10220.

Yao, L., Suzuki, H., Ozawa, K., Deng, J., Lehe, C., Fukamachi, H., Anderson, W. G., Kawakami, Y. and Kawakami, T. (1997) Interaction between protein kinase C and pleckstrin homology domains. Inhibition by phosphatidylinositol 4,5-bisphosphate and phorbol 12-myristate 13-acetate. *J. Biol. Chem.* **272**, 13033-13039.

Yao, L., Janmey, P., Friger, L. G., Han, W., Fujita, J., Kawakami, Y., Apgar, J. R. and Kawakami, T. (1999) Pleckstrin homology domains interact with filamentous actin. *J. Biol. Chem.* **274**, 19752-19761.