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Identification of p115 as a PLCγ1-binding protein and the role of Src homology domains of PLCγ1 in the vesicular transport☆

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Abstract

In order to gain further insight into the function(s) of PLCγ1, we tried to identify the binding partners that can interact with the SH223 domains of PLCγ1. Immunoscreening was performed with the purified antisera that are specific to SH223-binding proteins. Several immunoreactive clones were identified as the putative binding proteins and one of them was identified as p115. p115 was reported to be required for transcytotic fusion and subsequent binding of the vesicles to the target membrane. The interaction between PLCγ1 and p115 was specific to carboxyl-terminal SH2 domain and SH3 domain of PLCγ1, and also confirmed by biochemical approaches such as co-immunoprecipitation, pull-down assay, and glycerol gradient fractionation. To further characterize the role of SH domains of PLCγ1 in the vesicle transport pathway, secreted form of alkaline phosphatase (SEAP) reporter assay was carried out. When the SH2 and/or SH3 domains of PLCγ1 were deleted, the secretion of SEAP was significantly reduced. These findings indicate that the SH2 and SH3 domains of PLCγ1 may play a role(s) in the process of the vesicle transport via interaction with other vesicle-associated proteins such as p115.

Keywords: PLCγ1; p115; SH2; SH3; Binding protein

Inositol phospholipid-specific phospholipase C (PLC) is one of the primary regulatory enzymes in the receptor-mediated signaling pathway, which is involved in the regulation of many cellular events, including cell proliferation, differentiation, etc. [1]. The activated PLC catalyzes the hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP_2), generating two intracellular messengers, diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3), which in turn mediate the activation of protein kinase C (PKC) and intracellular Ca^{2+} ion release, respectively. Among the 11 mammalian PLC isozymes identified to date, only PLCγ isozymes have two SH2 and one SH3 domains that are located between the catalytic X and Y domains [2]. The SH2 and SH3 domains are shared by many signaling proteins involved in various cellular events such as mitogenesis through interaction with other proteins [3]. It has been shown that many proteins are involved in the PLCγ1-mediated signal transduction pathway through the interaction with SH2 and SH3 domains of PLCγ1. Previous reports documented that overexpression of SH223 domains of PLCγ1 could transform the rat fibroblast cells, suggesting that PLCγ1 may have an alternative signaling pathway through its SH domains to propagate mitogenic signals that are independent of its lipase activity [4]. However, the exact roles of SH domains of PLCγ1 in the cellular signal transduction pathway have not yet been elucidated.

In this report, we investigated the function of PLCγ1 by characterizing an important binding partner for its SH domains. To isolate the proteins that interact with SH domains of the PLCγ1, recombinant SH223 domains fused to glutathione *S*-transferase (GST) were incubated with the rat brain cytosolic fraction. These

☆ *Abbreviations:* PLC, inositol phospholipid-specific phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-triphosphate; PAGE, polyacrylamide gel electrophoresis; SEAP, secreted alkaline phosphatase.

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binding proteins were used as immunogen to raise the antisera. Using the immunoscreening method with the raised antisera, some proteins that have PLCγ1-binding properties were identified. One of them turned out to be p115 (transcytosis-associated protein (TAP) or general vesicular transport factor). p115 is involved in several transport steps including transcytotic vesicle to acceptor membrane [5], ER to Golgi [6], and transport between the Golgi stacks [7].

We report here an analysis of the specific interaction of p115 with carboxyl-terminal SH2 domain and SH3 domain of PLCγ1. Furthermore, the roles of the PLCγ1 in the regulation of the secretory pathway were examined by overexpressing the SH domain-deleted PLCγ1 mutants. When the SH2 and/or SH3 domain-deleted mutant forms of PLCγ1 were overexpressed, the secretion of SEAP, a reporter for secretory protein, was reduced. These results suggest that the SH223 domain of PLCγ1 is involved in the vesicle transport and fusion. Considering the fact that phosphoinositol metabolism is involved in the vesicular traffic in eukaryotic cells [8], our data regarding the association between PLCγ1 and vesicle traffic-related protein, p115, support this idea.

Materials and methods

Materials. Rat brains were purchased from Pel-Freez Biologicals (Rogers, AR). Mouse antibody against PLCγ1 was provided by Dr. Sue Goo Rhee (National Institutes of Health, USA). Glutathione–Sepharose 4B was purchased from Pharmacia (Piscataway, NJ) and all culture media were from Gibco-BRL (Gaithersburg, MD). Culture supplements for bacterial cells were purchased from Difco and nitrocellulose filter was from S&S. SEAP assay kit was purchased from Clontech (Palo Alto, CA). All other chemicals used in this study were obtained from Sigma (St. Louis, MS). Alkaline phosphatase- and horseradish peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG were purchased from Jackson Immuno Research (West Grove, PA).

Preparation of GST fusion proteins containing PLCγ1 SH domains and purification of binding proteins. Fusion proteins containing various domains of PLCγ1 were constructed and purified as described previously [9]. To isolate the SH223-binding proteins from rat brain cytosolic fraction, the rat brain homogenate that was prepared as described previously [10] was incubated with 1 mg fusion protein bound to glutathione–Sepharose 4B slurry. In vitro pull-down assay using GST fusion proteins, immunoprecipitation, and glycerol gradient assay were also carried out as previously described [10].

Preparation of antisera against the proteins that can associate with SH223 domain of PLCγ1 and isolation of cDNA clones by immunoscreening with these purified antisera. To raise the antibodies against the SH223-domain binding proteins, whole SH223-binding proteins were injected into two rabbits. The antibodies against SH223-binding proteins were precipitated with 50% (w/v) ammonium sulfate and purified with protein A–Sepharose column and GST–SH223 column, consecutively. To isolate the genes coding for proteins that can bind to SH223 domains of PLCγ1, 10^6 recombinant plaques from an oligo(dT)-primed λZAP II rat brain and mouse thymus cDNA expression libraries (Stratagene) were screened with the purified antisera that had been raised against SH223-binding proteins.

Preparation of polyclonal antibody against p115. Anti-serum against p115 was generated by immunizing the recombinant His_6-p115 fusion proteins into the rabbit. The cDNA fragment, corresponding to C-terminal parts (amino acids 235–959) of mouse p115 (nucleotides 712–2993, assuming the translation start site as +1), was inserted into pRSETB vector (Invitrogen) and overexpressed in *Escherichia coli* JM109. About 70 kDa His_6-p115 fusion protein was purified using Ni^{2+}–nitrilotriacetate (Ni–NTA)-agarose (Qiagen). Rabbits were immunized with about 0.5 mg His_6-p115 fusion protein. Anti-serum was adsorbed to an affinity chromatography column, eluted, dialyzed against PBS, and then used for immunoblot analysis.

Immunofluorescence microscopy. The cells were grown on glass coverslips, washed with ice-cold PBS, fixed with 3.7% paraformaldehyde for 1 h, and then permeabilized with blocking solution (1% bovine serum albumin in PBS) containing 0.3% Triton X-100 for 20 min on ice. Cells were incubated with polyclonal anti-p115 antibody and monoclonal anti-PLCγ1 antibody for 2 h at room temperature. Cells were then incubated with rhodamine-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories) for 2 h on ice. Stained cells were analyzed by fluorescence microscope (Nikon Optiphot).

Purification of recombinant p115 protein using baculovirus system. Mouse p115 cDNA was subcloned into the baculovirus expression vector pBacPACK (Clontech) and then modified to contain an NH_2-terminal His_6 tag for the purification of p115 protein. pBacPACK-p115 was transfected into Sf9 insect cells and recombinant p115 baculovirus particles were isolated and amplified according to the manufacturer's instructions (Clontech). Purified His_6-p115 was identified by Coomassie brilliant blue staining and immunoblot analysis using His_6 and p115 antibodies. Purified p115 was $> 99\%$ pure as determined by Coomassie staining.

Transfection and SEAP assay. The FLAG epitope-tagged expression constructs of PLCγ1 lacking SH2 and/or SH3 domain; [pFLAG-CMV2-wild, ΔSH22, ΔSH3, and ΔSH223] were provided by Suh and co-workers [11]. Transfection of mutant forms of PLCγ1 constructs, pSEAP and pGal, into PC12 cells was performed according to the manufacturer's protocol with LipofectAMINE PLUS (Gibco-BRL). The SEAP activity in the culture medium was measured according to the manufacturer's protocol (Clontech). Thirty hours after transfection, the culture medium (15 μl) was mixed with 60 μl assay buffer (Clontech) and 60 μl substrate (62.5 μM), and then incubated for 10 min. The chemiluminescence was then measured by luminometer (1420 Multilabel counter, Wallac). The transfection efficiency was measured by β-galactosidase assay using the cell lysate.

Result

To investigate the physiological functions of the SH2 and SH3 domains of PLCγ1, proteins that can bind to the recombinant SH domains fused to glutathione *S*-transferase (GST) were isolated from rat brain cytosolic fraction. For this purpose, we carried out immunoscreening method because this method using antibodies has a higher specificity than other methods in identifying new binding proteins. An affinity matrix was prepared by immobilization of GST–SH2–SH2–SH3 (GST–SH223) domain fusion protein on glutathione–Sepharose beads and then rat brain extracts were incubated. After incubation and extensive washing, various proteins were identified as binding partners when analyzed by SDS–PAGE (Fig. 1A). To raise the antisera against these binding proteins to be used for immunoscreening,

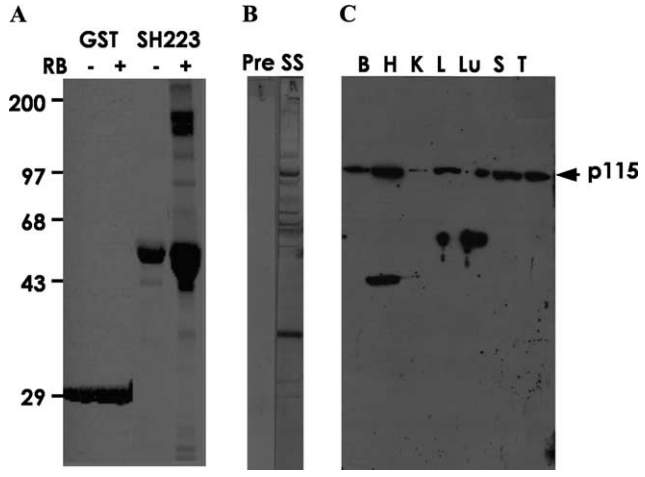

Fig. 1. Proteins from rat brain extracts bound to SH223 domains of PLCγ1. (A) Purified GST and GST-SH223 fusion proteins were bound to glutathione–Sepharose and incubated with Triton X-100 extracts of rat brain. The samples were washed with extraction buffer, separated on a 10% SDS–polyacrylamide gel, and then stained by Coomassie brilliant blue (RB: rat brain extract). (B) Western blotting with antisera which are used for immunoscreening. One hundred μg of rat brain extract was resolved by 10% SDS–PAGE and then transferred to NC membrane. The blot was probed with pre-immune serum (pre) or specific serum (SS) which have specificities to binding proteins. (C) Western blotting of p115 protein in various rat tissues. Total cytosolic proteins were prepared from various tissues of rat and separated on 10% SDS–PAGE (100 μg/lane). The blot was probed with affinity purified antibodies against bacterially expressed p115 protein (B: brain, H: heart, K: kidney, L: liver, Lu: lung, S: spleen, and T: thymus).

these SH-domain binding proteins from rat brain cytosol were pooled and then injected into rabbits. The Western blot analysis carried out with the raised and purified sera showed that these antibodies have broad specificities against the SH223-binding proteins (Fig. 1B). Through three rounds of immunoscreening with these purified antibodies, several immunoreactive clones were isolated. After nucleotide sequencing and analysis, it was found that nine of the 22 positive clones, with insert sizes ranging from 2.3 to 3.7 kb, were p115 cDNAs (GenBank Accession No. 096868) and two were synapsin IIb cDNAs (GenBank Accession No. 096867). In this study, we focused on the p115 cDNA as a promising target. To obtain a full-size cDNA clone for p115, mouse brain cDNA library was rescreened using semi-nested PCR method. The library was PCR amplified using a pair of primers (5′-GGGAGGGTTGGT GGGAGATCC-3′ for the first PCR and 5′-CTTCGGT CATCCAGTAACG-3′ for the second PCR) nested in the 5′ end of the longest clone and one primer in the Bluescript polylinker (BS2). Of the nine products that were sequenced, two contained the 5′ end of the mouse p115 cDNA. The mouse and rat nucleotide sequences are 92% identical and the predicted amino acid sequences are 98% identical (data not shown). For further experiments, anti-p115 antibody was generated using His_6-p115 as an antigen. This antibody recognizes a 115 kDa protein on immunoblot of various tissue cytosols from both mouse (data not shown) and rat (Fig. 1C) and a human origin cell line, A431 (Fig. 3C).

To confirm the interaction between p115 and SH domains of PLCγ1, rat brain extracts were incubated with recombinant GST-SH223 fusion proteins immobilized on Sepharose 4B. The bound proteins were then analyzed by Western blot with anti-p115 antibody. As shown in Fig. 2C, p115 can bind with GST fusion proteins containing the SH2 and SH3 domains of PLCγ1. PLCγ1 has two SH domains between the catalytic X and Y domains. Since p115 can bind with GST-SH22 domain of PLCγ1, it was tested which SH2 domain of PLCγ1, NH_2-terminal (N-SH_2) or COOH-terminal (C-SH2), is responsible for the interaction with this protein. GST fusion proteins which contain only one SH2 domain (N-SH2 or C-SH2) were incubated with rat brain extracts and the binding protein was analyzed by Western blotting. As shown in Fig. 2B, only the C-SH2 domain was able to interact with p115, in vitro. This result indicates that the two SH2 domains of PLCγ1 have different affinity to p115, and C-SH2 domain is responsible for the binding of PLCγ1 to p115. To verify that the PLCγ1-SH domain binds to p115 directly, His_6-p115 was overexpressed, purified in the baculovirus system, and then used for the direct binding test. Recombinant GST-SH fusion proteins were immobilized on glutathione–Sepharose, and incubated with purified

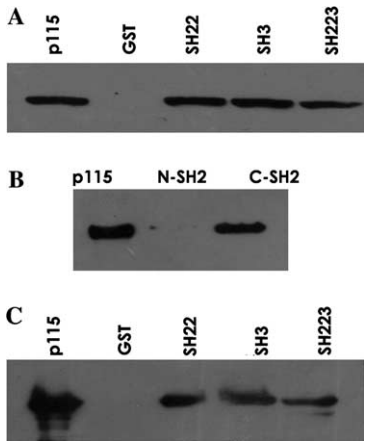

Fig. 2. Binding specificity of p115 to SH domains of PLCγ1. (A) Bacterially expressed GST fusion proteins containing SH-domains of PLCγ1 as indicated were immobilized on glutathione–Sepharose, washed, and incubated with rat brain cytosol extract. The bead samples were separated on 10% SDS–PAGE, and analyzed by immunoblotting with anti-p115 antibody. (B) Bacterially expressed GST fusion proteins containing NH_2-SH2 or COOH-SH2 domains of PLCγ1 were used for binding with p115 as in (A). (C) Direct interaction between purified p115 with SH-domains of PLCγ1. GST fusion proteins containing SH-domains of PLCγ1 were immobilized on glutathione–Sepharose, washed, and incubated with equal amounts of purified p115 from baculovirus overexpression system. The bead samples were separated on 10% SDS–PAGE and analyzed by immunoblotting with anti-His_6.

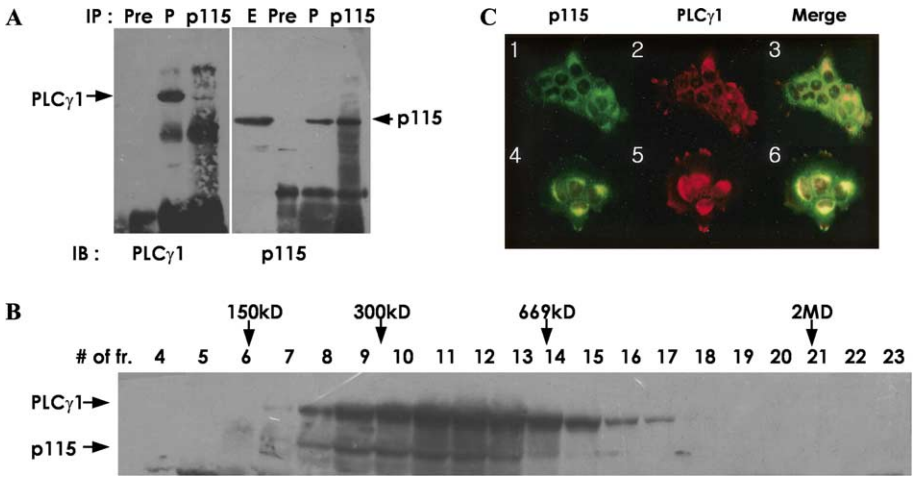

Fig. 3. p115 binds to PLCγ1 in vivo. (A) Immunoprecipitation and immunoblot analyses of p115 and PLCγ1. Triton X-100 extract of rat brain (E) was immunoprecipitated with anti-PLCγ1 (P), anti-p115, and non-immune rabbit IgG (Pre), respectively. The immunoprecipitates were detected with either anti-p115 or anti-PLC antibodies. (B) Co-fractionation of PLCγ1 and binding proteins in the glycerol gradient. Triton X-100 extracts of rat brain were fractionated in the linear glycerol gradient of 10–45% (v/v). After the ultracentrifugation at 12,000*g* for 36 h, each fraction was collected, precipitated with cold acetone, subjected to 10% SDS–PAGE, and analyzed by immunoblotting with anti-p115 and anti-PLC antibodies. (C) Immunofluorescence localization of p115 and PLCγ1 in A431 cell. A431 cells were grown on coverslips and washed with cold PBS. The cells were then fixed with paraformaldehyde and permeabilized with 0.3% Triton X-100. Cells were incubated with affinity purified antibodies against p115 and PLCγ1, followed by FITC-labeled goat anti-rabbit IgG (1, 4) and rhodamine-labeled goat anti-mouse IgG (2, 5), respectively.

p115. As seen in Fig. 2C, His_6-p115 fusion proteins were retained by GST-SH22, GST-SH3, and GST-SH223 fusion protein. These results indicate that associations between p115 and PLCγ1-SH domains are direct.

The in vivo interaction between p115 and PLCγ1 was also tested by coimmunoprecipitation from the 1% Triton X-100 lysate of the rat whole brain. Tissue lysate were immunoprecipitated with non-immune rabbit IgG, anti-PLCγ1, or anti-p115 antibodies, and then the resolved immunoprecipitates were probed with anti-p115 and anti-PLCγ1 antibodies, respectively. As shown in Fig. 3A, p115 was coimmunoprecipitated with PLCγ1. The glycerol-gradient fractionation was then carried out to confirm the in vivo interaction between p115 and PLCγ1. Rat brain was homogenized and fractionated on linear glycerol gradient of 10–45% (v/v). After the ultracentrifugation, the fractions were collected, subjected to SDS–PAGE, and then probed with anti-p115 and PLCγ1 antibodies. It has been reported that p115 is a homodimer with two globular heads and coiled-coil tails. p115 and 145 kDa PLCγ1 were detected in the fractions with the molecular mass range of 170–600 kDa and showed almost the same distribution (Fig. 3B), suggesting the possible interaction between p115 protein and PLCγ1 inside the cell.

The localizations of p115 and PLCγ1 in the cell were then examined in A431 human epidermoid carcinoma cell by immunofluorescence analysis. As previously reported [12], a clear and significant staining pattern was observed on one side of the perinuclear region, typical of the Golgi stacks, with anti-p115 antibody, even though the faint staining was also seen throughout the cytoplasm (Fig. 3C 1,4). This staining pattern can also be seen in various other cell lines such as NIH3T3 mouse fibroblast, L6 rat myoblast, and PC12 mouse pheochromocytoma neurocrine cell (data not shown). Double labeling of A431 cells with anti-PLCγ1 and polyclonal anti-p115 antibodies showed almost identical staining patterns (Fig. 3C 2,5). Taken together, these

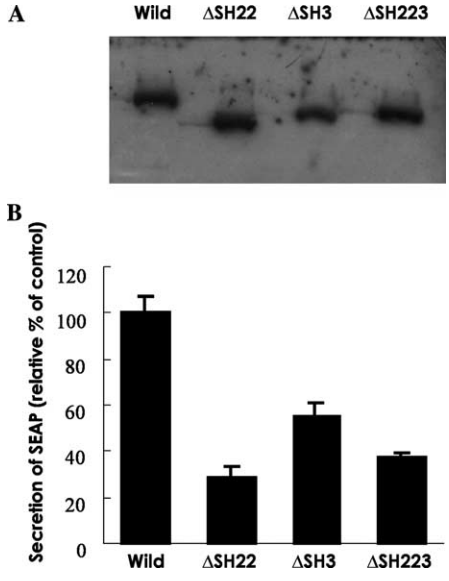

Fig. 4. The effect of the SH domains of PLCγ1 on the constitutive and regulated secretion. (A) A Western blot containing 50 μg of total protein from the cells harboring SH domain-deleted PLC constructs was probed with anti-Flag antibody. (B) PLCγ1 transfected PC 12 cells were cotransfected with pSEAP and pGal. After 30 h of incubation, the activity of SEAP in the culture supernatant was analyzed. The results were normalized with the activity of β-galactosidase and expressed as the percentage of SEAP activity found in the medium of control cells. The data are means (±SD) of three independent experiments.

results clearly demonstrated that p115 is colocalized with PLCγ1, predominantly near the Golgi apparatus region.

PLCγ1 is known as a key enzyme that can mediate the extracellular signals to the intracellular pathway; however, p115 is known to be involved in the vesicle transport. The interaction between these two proteins suggests that PLCγ1 may be involved in the vesicle transport pathway. To look at the role of the PLCγ1 in the regulation of the constitutive secretory pathway, the effect of overexpression of SH domain-deleted PLCγ1 was examined (Fig. 4). In this experiments, the secretion of secreted alkaline phosphatase (SEAP) was employed as a measure of constitutive secretory pathway [13]. When the SH domain-deleted mutant was overexpressed, the rate of vesicle transport or the secretion of SEAP was decreased. Especially, the ΔSH22 mutant was shown to have more inhibitory effects on the secretion of SEAP. These findings indicated that signal transduction through the SH2 domain of PLCγ1 may play a role in the vesicular transport and fusion via interaction with some other components of the secretory pathway.

Discussion

In this study, we exploited the function of PLCγ1 by characterizing an important binding partner for its SH domains. By immunoscreening approach, the p115, synapsin IIb and other novel proteins were identified as the PLCγ1-binding proteins. Synapsin is a protein that functions in synaptic vesicle formation, regulation of neurotransmitter release, and exocytosis of synaptic vesicles. It has been reported that synapsin can bind with SH3 domains of many proteins including PLCγ1, Src, and Grb2 [14]. However, the physiological meaning of these interactions remains to be determined. p115 is known to play a velcro factor or tethering protein because it is required for endoplasmic reticulum to Golgi traffic, intra-Golgi transport [12], binding of transcytotic vesicles to acceptor membranes [5], and regrowth of Golgi cisternae from post-mitotic fragments, etc. [15]. p115 is located on the Golgi membrane via direct interaction with GM130 and giantin [16] and also found in the vesicular–tubular cluster [6]. In our experience, PLCγ1 is also resident in the Golgi part of the cell (Fig. 3C). The fact that p115 and synapsin IIb can bind with PLCγ1 suggests that PLCγ1 may be involved in the regulation of the intracellular vesicle transport such as exocytosis, neurotransmitter release, and vesicle transport from ER to Golgi. To elucidate the meaning of the interaction of p115 with PLCγ1, the activity of PLCγ1 was measured in vitro. This interaction, however, did not have any effects on the activities of PLC β, γ, and δ isozymes (data not shown). However, the possibility still exists that the activity of PLCγ1 is modulated by p115 indirectly via interaction with other effectors in vivo.

Previous studies have suggested that phosphoinositol plays critical roles in the regulation of membrane traffic. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is not only the substrate of PLCγ1 but also the regulator of the actin cytoskeleton assembly and vesicle trafficking [8]. It has been reported that formation of constitutive secretory vesicles at the *trans*-Golgi network (TGN) is stimulated by a PLD whose activity in turn is regulated by a small G protein ADP-ribosylation factor (ARF), PKCα and PIP_2 [17]. Previous report has suggested that the hydrolysis of PIP_2, by IPPases (inositol polyphosphate phosphatases) such as synaptojanin and OCRL [18], might constitute a common mechanism for inhibition of PLD during membrane invagination and uncoating. We previously described that synaptojanin and AP180 can bind with SH2 domain of PLCγ1 and inhibit PLCγ1 activity [9,10]. Jones and Carpenter [19] have shown that PLCγ enzymes were greatly stimulated by phosphatidic acid (PA) in vitro. PA is primarily derived from phosphatidylcholine by the action of PLD. In this scheme, the PLD would provide PA to activate the PLCγ which in turn hydrolyze the PIP_2 to down-regulate the PLD activity. Perhaps DAG and PKC, which are generated by PLCγ1, could regulate the transport of vesicles from ER to Golgi by modulating the PLD activity. However, the molecular mechanisms of such communication are presently unknown. Godi et al. [20] showed that the increased PIP_2 enhanced the recruitment of βIII spectrin and actin to Golgi membrane. As a result, the mash of Golgi membrane skeleton was changed to allow the fusion and fission of vesicles. It is known that proteins which link the actin cytoskeleton to membrane surfaces, including vinculin [21] and α-actinin [22] or that sever actin filaments, like gelsolin [23,24], bind PIP_2 and negatively modulate the PLC. PLC might play critical roles in the vesicle transport by modulating and regulating the contents of PIP_2 in the Golgi membrane via interaction with other proteins including p115.

There is another evidence to suggest that lipid-modifying enzymes are involved in the ER to Golgi transport pathway through the interaction with p115. It has been reported that p115 could bind to CCT (CTP: phosphocholine cytidylyltransferase) and regulate its activity [25]. It is observed that activated PLC stimulates the CCT activity and the synthesis of PC (phosphatidylcholine) in membranes is inhibited when PLC inhibitors were treated [26]. The changes of PC contents in the membranes cause an alteration in the physical form of phospholipid at specific membrane domains. These data suggest that p115 might be the connecting protein to link the vesicle transport and biosynthesis of phosphatidyl lipid by concentrating lipid-modifying enzymes such as CCT and PLCγ1 at specific subregions of the plasma membrane.

Several interconnections have emerged between PI (phosphoinositide) metabolism and some of the GTPases (such as ARF and Rabs) or their accessory proteins, which suggests that at least some of the actions of PIs may involve GTPase. The possible interaction between PIs and Rab proteins was suggested by the study that PI3K binds to Rab5 in the endosome to generate the binding site for EEA [27]. It has also been shown that the recruitment of p115 to membranes is mediated by Rab1 [28]. Likewise, there is a possibility that PLCγ1 may bind to Rab1 and p115 complexes to facilitate the interaction of other effectors by changing the phosphatidylinositol contents in the transport vesicle. Another possibility concerning the roles of PLCγ1 in the vesicle transport is that the SH domains of PLCγ1 have another enzyme activity. Recent work has shown that SH3 domain of PLCγ1 functions as GEF (guanine nucleotide exchange factor) for small nuclear GTPase protein PIKE (phosphatidylinositol-3-OH kinase enhancer) [29]. If SH3 domain of PLCγ1 functions as GEF for Rab1, p115 makes SH3 domains of PLCγ1 exchanging the nucleotide of Rab1 by recruiting the PLC. However, SH3 domain of PLCγ1 does not work as a GEF for Rab1 protein in our experience (data knot shown).

In this study, p115 was shown to interact with PLCγ1 via C-SH2 and SH3 domains (Fig. 2). There are several bodies of evidence to suggest that SH3 domains play a critical role in vesicular trafficking [30]. In previous studies, the lipid-modifying enzyme, synaptojanin, and endocytosis-related enzyme, AP180, could also bind to PLCγ1 via C-SH2 domains [9,10]. Since, the N- and C-SH2 domains of PLCγ1 have different specificities to its binding partners, it seems likely that N-SH2 is involved in the signal transduction pathway and C-SH2 domain and SH3 domain are involved in the vesicle transport pathway. In this study, search for a plausible role of PLCγ1 other than the conventional signal transduction leads to the interesting possibility that PLCγ1 and p115 may act in communication between phosphatidylinositol metabolism and vesicular transport.

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