Structure and Dynamics of the Phospholipase C-δ1 Pleckstrin Homology Domain Located at the Lipid Bilayer Surface*

Satoru Tuzi†, Naoko Uekama, Masashi Okada, Satoru Yamaguchi, Hazime Saitō, and Hitoshi Yagisawa

From the Department of Life Science, Himeji Institute of Technology, Harima Science Garden City, Kouto 3-chome, Kamigori, Hyogo 678-1297, Japan

Received for publication, January 6, 2003, and in revised form, April 28, 2003
Published, JBC Papers in Press, May 7, 2003, DOI 10.1074/jbc.M300101200

Despite the importance of signal transduction pathways at membrane surfaces, there have been few means of investigating their molecular mechanisms based on the structural information of membrane-bound proteins. We applied solid state NMR as a novel method to obtain structural information about the phospholipase C-δ1 (PLC-δ1) pleckstrin homology (PH) domain at the lipid bilayer surface. NMR spectra of the alanine residues in the vicinity of the β5/β6 loop in the PH domain revealed changes in local conformations due to the membrane localization of the protein. We propose that these conformational changes originate from a hydrophobic interaction between the amphipathic α-helix located in the β5/β6 loop and the hydrophobic layer of the membrane and contribute to the membrane binding affinity, interdomain interactions and intermolecular interactions of PLC-δ1.

Pleckstrin homology (PH) domains are well defined structural modules of about 120 amino acid residues (1, 2) mainly found in proteins involved in cellular signaling and cytoskeletal functions (3–6). It has been proposed that these domains function as mediators of intermolecular interactions analogous to many other structural modules involved in cellular signaling (e.g. SH2 and SH3 domains). Many kinds of inositol lipids and inositol phosphates have been identified as important ligands of PH domains (3–6), and, in some cases, the PH domains also interact with other proteins and mediate protein-protein interactions (5).

The PH domain of phospholipase C-δ1 (PLC-δ1) is one of the most extensively studied PH domains. It has been proposed that it regulates the membrane localization of PLC-δ1 (7, 8) through its high affinity specific interaction with phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2), a PLC-δ1 ligand (9), and di-myoinositol 1,4,5-trisphosphate (Ins(1,4,5)P3) (10), a product of PtdIns(4,5)P2 hydrolysis by PLC-δ1. Despite the rather low sequence similarity among the PH domain families, the secondary and tertiary structural motifs of the PH domain are highly conserved (3–6). A high resolution structural model of the rat PLC-δ1 PH domain forming a complex with Ins(1,4,5)P3 has been determined by x-ray diffraction study at 1.9-Å resolution (11). The model consists of a seven-stranded β sandwich formed by two orthogonal anti-parallel β-sheets and a C-terminal amphipathic α-helix. These are conserved structural motifs among the PH domains whose structures have been determined by x-ray diffraction and NMR studies. The loops between the β-strands, particularly the β1/β2, β3/β4, and β6/β7 loops, differ greatly among the PH domains, and, in the case of the PLC-δ1 PH domain, the β1/β2 and β3/β4 loops mainly interact with Ins(1,4,5)P3. The β5/β6 loop of the PLC-δ1 PH domain includes a characteristic short amphipathic α-helix (α2-helix) that is not found in other PH domain model structures studied so far.

Because functionally important intermolecular interactions of PLC-δ1 with its ligand, PtdIns(4,5)P2, or other proteins included in the signal transduction pathways (e.g. transglutaminase II (Go)1) take place at the membrane surface (12–14), structural information of PLC-δ1 at the membrane surface is indispensable for understanding the molecular mechanism underlying the functions of PLC-δ1. The conformation and dynamics of peripheral membrane proteins at the lipid bilayer surface are expected to be different from those in solution, due to intermolecular interactions between the protein and lipids, changes in pH and ionic strength induced by surface charges of the membrane, and drastic changes in the dielectric constant at the lipid bilayer surface.

Despite the importance of the PLC-δ1 structure at the lipid bilayer surface, there is virtually no structural information about the peripheral membrane proteins at the membrane surface, due to a lack of means of investigating the molecular structure of proteins at the lipid bilayer surface at atomic resolution. In this study, we applied solid state NMR as a novel method of gaining insights into atomic level structural information of a peripheral membrane protein at the membrane surface under conditions similar to those of natural membranes. Solid state NMR is a highly suitable technique for this purpose, because it can provide information about the conformation and dynamics of individual amino acid residues in an intact protein under a wide variety of conditions, including those in a protein-lipid vesicle complex suspended in buffer at ambient temperature. By metabolic introduction of carbon-13-labeled alanine residues into a protein as NMR probes, the local conformation and dynamics of selectively labeled amino acid residues are readily analyzed (15–19). Here, we applied this “site-directed” high resolution solid state 13C NMR technique to the PH domain of PLC-δ1 as the first trial of a high resolution solid state NMR investigation of peripheral mem-
brane proteins involved in lipid signal transduction pathways and obtained evidence for the conformational change of the PH domain at the membrane surface.

EXPERIMENTAL PROCEDURES

Materials—Phosphatidylycerine (PtdCho) from bovine liver was purchased from Avanti Polar Lipids (Birmingham, AL). PtdIns(4,5)P$_2$ was from Sigma (St. Louis, MO). L-[3-$^{13}$C]Alanine was from CIL (Andover, MA). All reagents were used without further purification.

Expression Vector and Site-directed Mutagenesis—The cDNA encoding rat aortic PLC-$\xi$-1 fragment (1–140) was subcloned into a pGEX-2T-based bacterial expression vector (pGEX-2T from Amersham Biosciences), designated pGST3. Individual point mutations were introduced into the plasmid pGST3-PLC-$\xi$-1 (141–768) encoding the PH domain of the wild type enzyme by T4 DNA polymerase-based mutagenesis using a Transformer<sup>TM</sup> site-directed mutagenesis kit (Clontech). The selection primer was (5'-GGTTTCTTAGTCGACAGGT-3') and -ELGPRPNWPTS, at the N and C termini of the natural amino acid sequence of the wild type PLC-$\xi$-1 PH domain-PtdCho/PtdIns(4,5)P$_2$ vesicle complexes were preformed as relative shifts from the TMS value. Dynamic light scattering measurement indicated that the PH domain containing 1% diethiothreitol and 0.025% Na$_2$S$_2$O$_4$ (molar ratio of PtdCho/PtdIns, and PH domain was 40:2:1) and incubated for 20 min at 4 °C to allow complex formation. The protein-vesicle complexes were concentrated by ultra-centrifugation (541,000 × g for 6 h at 4 °C). The [3-$^{13}$C]Ala-labeled PLC-$\xi$-1 PH domain-Ins(1,4,5)P$_3$ complex was prepared by mixing the PH domain and Ins(1,4,5)P$_3$ solutions (10 mM MES buffer (pH 6.5) containing 25 mM NaCl, 10% glycerol, and 0.025% Na$_2$S$_2$O$_4$; the molar ratio of the PH domain and Ins(1,4,5)P$_3$ was 1:1:1). The PH domain-Ins(1,4,5)P$_3$ complex solution was concentrated by ultrafiltration using Microcon YM-3 (Amicon). The [3-$^{13}$C]Ala-labeled PH domain-PtdCho/ PtdIns(4,5)P$_2$ vesicle complexes and the [3-$^{13}$C]Ala-labeled PH domain-Ins(1,4,5)P$_3$ complex were placed in a 5-mm outer diameter zirconia probe under solid state NMR sample rotor and sealed with epoxy resin to prevent evaporation of water.

Measurement of Solid State $^{13}$C NMR Spectra—High-resolution solid state $^{13}$C NMR spectra were recorded on a Varian Infinity 400 spectrometer (13C: 100.6 MHz), using cross polarization-magic angle spinning (CP-MAS) and single pulse excitation dipolar decoupled-magic angle spinning (DD-MAS) methods. The spectral width, acquisition time, and repetition time for CP-MAS and DD-MAS experiments were 40 kHz, 50 ms, and 4 s, respectively. The contact time for the CP-MAS experiment was 1 ms. Free induction decays were acquired with 2,048 data points and Fourier-transformed as 32,768 data points after 30,720 data points were zero-filled. The rf pulses for carbon and protons were 5.0 μs, and the spinning rates were 4 kHz. The dipolar decoupling field strength was 65 kHz unless indicated otherwise in the text. Transients were accumulated 20,000–40,000 times until a reasonable signal-to-noise ratio was achieved. The $^{13}$C chemical shifts were referenced to the carboxyl signal of glycine (176.03 ppm from tetramethylsilane (TMS)) and then expressed as relative shifts from the TMS value.

Measurement of Dynamic Light Scattering—Dynamic light scattering of the wild-type PH domain-Ins(1,4,5)P$_3$ complex in 10 mM MES buffer (pH 6.5) containing 25 mM NaCl, 10% glycerol, and 0.025% Na$_2$S$_2$O$_4$ was measured at 20 °C using a Dyna Pro dynamic light scattering/molecular sizing instrument (Protein Solutions). The molecular weights of the monomeric or oligomeric PLC-$\xi$-1 PH domain particles were estimated from the particle sizes by assuming the particles had a spherical shape.

RESULTS

NMR Spectra of [3-$^{13}$C]Ala Residues in the PLC-$\xi$-1 PH Domain-Ins(1,4,5)P$_3$ Complex—Fig. 2 (A and B) shows NMR spectra of the [3-$^{13}$C]Ala-labeled PH domain-Ins(1,4,5)P$_3$ complex in solution measured using the single pulse excitation dipolar decoupled-magic angle spinning (DD-MAS) method using different amplitudes of magnetic fields for dipolar decoupling: 19 and 55 kHz. Five narrow peaks resonating at 14.55, 15.35, 15.77, 17.49, and 18.46 ppm in Fig. 2A, which are unaffected by dipolar decoupling field strength, are ascribed to the $^{13}$C-labeled side-chain methyl groups of five alanine residues in the monomeric or oligomeric PH domain. The dipole-dipole interactions between $^1$H and $^{13}$C nuclei that cause serious increases in the line width of $^{13}$C signals are decoupled by fast isotropic rotational motions of the small PH domain particles. A peak at 11.41 ppm, indicated by an asterisk, is thought to be an artifact because of the lack of reproducibility. An obvious increase in line width of the peak at 16.79 ppm under the weaker decoupling field (Fig. 2A) indicates that this signal arises from larger complexes with different particle sizes in solution. A major component consists of particles with a hydrodynamic radius greater than 10 nm and an average molecular mass between 19 and 50 kDa. A minor component consists of particles with a hydrodynamic radius greater than 10 nm and an average molecular mass greater than 50 MDa. The former component is thought to comprise the monomeric, dimeric, and/or trimeric PH domains, and the latter aggregated represents clusters of PH domains consisting of a large number of molecules. These results support...
the above-mentioned assignments of the signals to particles with different sizes. Although the large aggregated clusters undergo slow rotational motions, these motions are sufficiently fast to eliminate most of the dipole-dipole interaction between $^1$H and $^{13}$C nuclei that is required to form magnetization through cross-relaxation between $^1$H and $^{13}$C nuclei, because no signal was observed using the cross-polarization-magic angle spinning (CP-MAS) method (data not shown).

Studies of synthetic polypeptides, structural proteins, and membrane proteins have shown that the chemical shift of the $C_\beta$ carbon in the Ala residue in high resolution solid state $^{13}$C NMR is primarily determined by the torsion angles ($\phi, \psi$) of the main chain of the residue itself (21–23). The narrow line widths and the symmetric line shape of the peaks from the smaller particles in Fig. 2A reveal that the conformation of the Ala residues in the monomeric PH domain and dimer and/or trimer, if any, observed by NMR are identical, showing no structural variation that causes displacement of chemical shifts. The buffer composition of the [3-$^{13}$C]Ala-labeled PH domain-Ins(1,4,5)P$_3$ complex solution was the same as the crystallization buffer used in the x-ray diffraction study in which the random coil of the C terminus of bacteriorhodopsin are shown by vertical bars at the bottom of the spectra. The peak indicated by an asterisk is an artifact.

High Resolution Solid State NMR Spectra of the PH Domain-PtdIns(4,5)P$_2$ Complex—Fig. 3 (A and B) show DD-MAS and CP-MAS NMR spectra of the [3-$^{13}$C]Ala-labeled PH domain forming a complex with PtdIns(4,5)P$_2$ in the PtdCho/ PtdIns(4,5)P$_2$ liposome suspended in buffer solution at 20 °C. The chemical shifts of the signals from the [3-$^{13}$C]Ala-labeled PLC-$\delta_1$ PH domain-Ins(1,4,5)P$_3$ complex are indicated by asterisks. The chemical shifts of the peaks of Ala residues in the [3-$^{13}$C]Ala-labeled PLC-$\delta_1$ PH domain-Ins(1,4,5)P$_3$ complex are shown by vertical bars at the bottom of the spectra and dotted lines.

**Fig. 2.** DD-MAS $^{13}$C NMR spectra of the [3-$^{13}$C]Ala-labeled PLC-$\delta_1$ PH domain-Ins(1,4,5)P$_3$ complex measured with different dipolar decoupling field strengths (A, 19 kHz; B, 55 kHz) at 20 °C. The chemical shifts of the peaks are shown at the top of the spectra. The chemical shifts of the typical $\alpha$-helix and $\beta$ sheet of poly-$L$-alanine and of the random coil of the C terminus of bacteriorhodopsin are shown by vertical bars at the bottom of the spectra. The peak indicated by an asterisk is an artifact.

**Fig. 3.** DD-MAS (A) and CP-MAS (B) NMR spectra of the [3-$^{13}$C]Ala-labeled PLC-$\delta_1$ PH domain-PtdIns(4,5)P$_2$ complex measured at 20 °C. The peaks arising from lipid molecules are indicated by asterisks. The chemical shifts of the peaks of Ala residues in the [3-$^{13}$C]Ala-labeled PLC-$\delta_1$ PH domain-Ins(1,4,5)P$_3$ complex are shown by vertical bars at the bottom of the spectra and dotted lines.
ppm in the DD-MAS NMR spectrum (Fig. 3A) are very similar to those of the peaks observed for the PH domain-Ins(1,4,5)P$_3$ complex (14.55, 15.35, and 15.77 ppm; Fig. 2A). In contrast to these signals, the signals resonated at 17.49 and 18.46 ppm in the spectra of the PH domain-Ins(1,4,5)P$_3$ complex (Fig. 2A) show an upfield displacement to 16.99 ppm and a downfield displacement up to 19.1 ppm, respectively. To assign these signals, mutant PH domains in which Ala residues are replaced by Gly or Leu residues are prepared. Fig. 4 (A and B) show the CP-MAS NMR spectra of the [3-13C]Ala-labeled A112G mutant PH domain in which Ala-112 is replaced by Gly and A88G mutant PH domain in which Ala-88 is replaced by Gly forming complexes with PtdCho/PtdIns(4,5)P$_2$ vesicles, respectively. The signals resonating between 18.5 and 19.1 ppm (Figs. 3B and 4A) were assigned to Ala-112 based on the disappearance of these peaks in the spectrum of A112G. The replacement of Ala-112 by Gly induced downfield displacement of the peak at 16.99 ppm in the CP-MAS spectrum of the wild type PH domain (Fig. 3B) to 17.50 ppm (Fig. 4B). The peaks at 15.37 and 15.83 ppm in the CP-MAS NMR spectrum of the wild type PH domain (Fig. 3B) were also shifted in the range of 15.51 and 16.40 ppm (Fig. 4B). Fig. 4C shows the CP-MAS NMR spectrum of the [3-13C]Ala-labeled A88G mutant PH domain. The peak at 16.99 ppm in the CP-MAS spectrum of the wild type PH domain (Figs. 3B and 4A) was assigned to Ala-88, because this peak disappeared in the spectrum of A88G (Fig. 4C). Fig. 5 (A–C) shows the DD-MAS NMR spectra of A116L, A118G, and A21L mutant PH domain-PtdCho/PtdIns(4,5)P$_2$ vesicle complexes, respectively. The signals resonating at 15.83 and 15.40 ppm were ascribed to Ala-116 and Ala-118 based on the disappearances of the peaks indicated by arrows in the spectra of A116L (Fig. 5A) and A118G (Fig. 5B), respectively. As shown by a closed triangle in Fig. 5B, a strong suppression of the signal of Ala-112 was induced by the replacement of Ala-118 by Gly. This suppression would be caused by decrease in an efficiency of the dipole decoupling due to an interference between the frequency of the dipole decoupling field (55 kHz) and a newly induced thermal motion of the Ala-112 residue at a frequency around 10$^4$-10$^5$ Hz (25). The removal of the side-chain methyl group of Ala-118 could facilitate such a thermal motion of Ala-112, because the side chain of Ala-118 is in van der Waals contact with the side chain of Ala-112 in the three-dimensional model structure. Fig. 5C shows the DD-MAS spectrum of A21L mutant PH domain-PtdCho/PtdIns(4,5)P$_2$ vesicle complex. A remarkably intense signal at 16.9 ppm indicated by an open triangle was ascribed to the random coil structure of the denatured PH domain included in the aggregated cluster similar to that observed for the PH domain-Ins(1,4,5)P$_3$ complex (Fig. 2). The replacement of Ala-21 might reduce a stability of the PH domain. Because the peaks at 15.83 and 15.40 ppm remained intact in the spectrum of A21L, the peak resonating at 14.41 ppm in the CP-MAS spectrum of the wild type PH domain-PtdCho/PtdIns(4,5)P$_2$ vesicle complex (Fig. 3B) could be ascribed to Ala-21, although an effort of direct assignment from a removal of the peak by the replacement of Ala-21 was unsuccessful due to the intense signal of the methyl carbon of the lipid resonated at 14.20 ppm either in the DD-MAS or CP-MAS spectra of the A21L mutant PH domain-PtdCho/PtdIns(4,5)P$_2$ vesicle complex.
due to the improved cross-polarization efficiency caused by originating from the phospholipid methyl groups is enhanced. In the CP-MAS NMR spectrum at 4 °C, a peak at 14.27 ppm and N-terminal the binding of the PH domain to PtdIns(4,5)P2 in liposomes. type [3-13C]Ala-labeled PLC-
(A) indicates a significant conformational spectrum (Fig. 3
B) observed for the peak at 16.99 ppm in the CP-MAS spectrum (Fig. 3
A) compared with that in the DD-
MAS spectrum (Fig. 3
B) dotted lines). indicates that the Ala-112 taking the A
B domain-PtdCho/PtdIns(4,5)P2 vesicle complex measured at 4 °C (solid lines) and 20 °C (dotted lines). The peaks arising from lipid molecules are indicated by asterisks.

The similarity in chemical shifts of Ala-116, Ala-118, and Ala-21 between the PH domain-Ins(1,4,5)P3 complex and the PH domain-PtdCho/PtdIns(4,5)P2 vesicle complex indicates that the conformations of the Ala residues contained in the C- and N-terminal α-helices of the PH domain are not affected by the binding of the PH domain to PtdIns(4,5)P2 in liposomes. Conversely, the upfield displacement of 0.50 ppm from 17.49 ppm in the spectrum of the PH domain-Ins(1,4,5)P3 complex (Fig. 2A) observed for the peak at 16.99 ppm in the CP-MAS spectrum (Fig. 3B) indicates a significant conformational change of the Ala-88 in the β5/β6 loop. The slight upfield displacement of this signal from 16.99 ppm in the CP-MAS NMR spectrum to 16.89 ppm in the DD-MAS NMR spectrum (Fig. 3A) could be the result of a minor contribution to the signal from a small amount of the aggregated cluster of the denatured PH domain observed at 16.79 ppm in the DD-MAS NMR spectra (Fig. 2, A and B). The broad signal resonating between 18.5 and 19.4 ppm in the DD-MAS and CP-MAS NMR spectra of the PH domain-PtdCho/PtdIns(4,5)P2 vesicle complex (Fig. 3, A and B), corresponding to the peak of the PH domain-Ins(1,4,5)P3 complex at 18.46 ppm (Fig. 2A), is attributed to the coexistence of a variety of different conformations of Ala-112 at the C terminus of the β7 strand. Notably, the higher relative signal intensity of the peak at 18.82 ppm in the DP-MAS NMR spectrum (Fig. 3B) compared with that in the DD-
MAS spectrum (Fig. 3A) indicates that the Ala-112 taking the conformation corresponding to this peak is highly immobile.

Fig. 6 (A and B) shows the DD-MAS and CP-MAS NMR spectra of the PH domain-PtdCho/PtdIns(4,5)P2 vesicle complex, respectively, at 4 °C (solid trace) and 20 °C (dotted trace). In the CP-MAS NMR spectrum at 4 °C, a peak at 14.27 ppm originating from the phospholipid methyl groups is enhanced due to the improved cross-polarization efficiency caused by decrease in mobility of the lipid molecules at 4 °C (Fig. 6B). In the DD-MAS spectrum at 4 °C, the relative intensity of the Ala-112 signal increased to form a new peak at 18.78 ppm (Fig. 6A). In the CP-MAS spectrum at 4 °C, the line width of the Ala-112 signal resonating at 18.77 ppm is narrower than that in the spectrum at 20 °C (Fig. 6B). These changes indicate that the conformation of Ala-112 corresponding to the chemical shift of 18.77–18.78 ppm, which is most immobile at 20 °C, becomes dominant at 4 °C.

**DISCUSSION**

In this study, we aimed to gain insights into the structural features of the PH domain at the lipid bilayer surface when the domain forms a complex with PtdIns(4,5)P2. The PH domain of PLC-δ1 forms a high affinity complex with either PtdIns(4,5)P2 or Ins(1,4,5)P3 with comparable binding constants (10) through a specific interaction between the phosphoinositol group and the side chains located at the basic surface of the PH domain (e.g. the β1/β2 and β3/β4 loops) (11). There is a possibility, however, that additional interactions between the PH domain and the lipid bilayer contribute to the characteristics of the PH domain at the membrane surface, such as binding affinity, conformation, mobility, and orientation. Such nonspecific interactions might be responsible for the dependence of binding affinity of the PH domain to inositol compounds on assay conditions, such as pH, ionic composition of buffer, or lipid composition of membrane (4).

Possible candidates for such interactions are electrostatic interactions between the positively charged surface of the PH domain, which is not involved in the specific interaction with PtdIns(4,5)P2 and the lipid head groups, and hydrophobic interactions between the hydrophobic surfaces of the PH domain and the hydrophobic inner layer of the lipid bilayer. These interactions, if any, would modify the conformation, dynamics, and orientation of the PH domain at the lipid bilayer surface, which in turn, would influence lipid-protein and protein-protein interactions at the membrane surface involved in signal transduction pathways. In the case of multidomain proteins such as PLC-δ1, which contains the PH domain, EF-hands, an active site domain and a C2 domain, intramolecular interaction between the domains would also be affected by the conformational characteristics of the domains at the membrane surfaces.

As shown in Fig. 3, changes in the 13C NMR spectra of the [3-13C]Ala-labeled PLC-δ1 PH domain clearly revealed conformational changes induced by localization of the domain at the surface of the PtdCho/PtdIns(4,5)P2 vesicle. The changes in the PH domain structure were found to occur in the vicinity of the β5/β6 loop containing Ala-88 and the C terminus of the β7 strand containing Ala-112. In contrast, the N- and C-terminal α-helices located at the surface opposite to the ligand binding site of the PH domain showed virtually no conformational change.

The side chains of Leu-84 and Ala-88 in the β5/β6 loop and Ile-111 in the β7 strand form a hydrophobic cluster between the α-helix in the β5/β6 loop (α2-helix; Fig. 7A) and the β7 strand according to the three-dimensional model structure of the PH domain-Ins(1,4,5)P3 complex (11). The proximity of Ala-88 and Ala-112 in the model structure suggests a relationship between these residues in the conformational changes. The origin of the upfield displacement of the Ala-88 signal is ascribed to a direct interaction between the α2-helix and the membrane, considering the close location of the β5/β6 loop to the positively charged lipid-binding surface of the PH domain (Fig. 7A). Taking into account the highly amphipathic nature of the α2-helix (as shown in Fig. 7B by a helical wheel), it is plausible to predict a hydrophobic interaction between the hydrophobic face of the α2-helix and the hydrophobic inner layer of the lipid bilayer. In fact, x-ray diffraction studies of membrane binding states of synthetic model peptides (26, 27) have suggested that such a hydrophobic interaction facilitates the orientation of an am-
The lipid bilayer is colored a membrane surface, suggests the formation of an additional membrane that might cause a tilt of the PH domain.

Because only changes of three torsion angles, \( \phi \) and \( \varphi \) of Arg-95, are required for the reorientation of the amide group of Arg-95, this reconstitution of the hydrogen bond is expected to occur readily. The chemical shift of the Ala-112 signal at 4 °C (18.77-18.78 ppm; Fig. 6) reflects the conformation with the lowest energy at the membrane surface that might include the hydrogen bond between Arg-95 and Ala-112. Another reason for the origin of the conformational change is the C terminus of the \( \beta \) strand is interaction between the \( \beta \) strand and the main chains of the \( \beta \)6 and \( \beta \)7 strands.

The non-specific hydrophobic or electrostatic interactions described above would contribute to the affinity of the PLC-\( \delta 1 \) PH domain to PtdIns(4,5)P\(_2\) in the membrane. These auxiliary mechanisms for membrane binding are probably susceptible to assay conditions, such as the structures of the hydrophobic acyl-chains of the lipids, surface charges of the membrane, pH value, and ionic strength of the buffer. For example, although it has been reported that the \( K_D \) value of the PLC-\( \delta 1 \) PH domain-PtdIns(4,5)P\(_2\) interaction measured using dimyristoyl phosphatidylcholine (DMPC)-PtdIns(4,5)P\(_2\) vesicles is 1.66 \( \mu \)M, eight times larger than that of the PLC-\( \delta 1 \) PH domain-Ins(1,4,5)P\(_3\) interaction (210 nM) (10), the affinity of the PLC-\( \delta 1 \) PH domain to PtdIns(4,5)P\(_2\) in the natural membrane could be different and might be higher than that observed for the DMPC vesicle system due to the presence of unsaturated acyl chains.

Although more detailed structural information about the membrane binding state of the PH domain is required to judge the validity of these models, the results of the solid state NMR experiments clearly indicate that the PLC-\( \delta 1 \) PH domain has an unique conformation and dynamics at the lipid bilayer surface, which are different from those in solution. The structural information at the membrane surface is indispensable for gaining insights into the molecular mechanisms of the functions of peripheral membrane proteins. Our results also proved that

**FIG. 7.** The possible conformational change of the PLC-\( \delta 1 \) PH domain at the membrane surface. **A**, a schematic representation of the PLC-\( \delta 1 \) PH domain-Ins(1,4,5)P\(_3\) complex, based on the three-dimensional model structure determined by x-ray diffraction study (11). The \( \beta \)7 strand in contact with the \( \alpha \)2-helix is highlighted in yellow. The positions of the Ala residues are indicated as green circles. **B**, the \( \alpha \)2-helix is represented as a wheel viewed from the C terminus. Residues with hydrophobic and polar side chains are colored blue and red, respectively. **C**, a schematic model of the changes in conformation and orientation of the PLC-\( \delta 1 \) PH domain induced at the membrane surface. The hydrophobic and hydrophilic surfaces of the amphipathic \( \alpha \)2-helix are colored blue and red, respectively. The \( \beta \)7 strand is colored blue. The lipid bilayer is colored a purple shade. The re-orientation of the \( \alpha \)2-helix induces hydrophobic interaction between the hydrophobic surfaces of the \( \alpha \)2-helix and \( \beta \)7 strand and the hydrophobic inner layer of the membrane that might cause a tilt of the PH domain.

**FIG. 8.** A possible conformational change in the vicinity of Ala-112 at the membrane surface. The conversion and hydrogen bonds proposed in the model structure (11) (left) could change into the structure containing a newly formed hydrogen bond between Arg-95 and Ala-112 and the consequently elongated \( \beta \)6 and \( \beta \)7 strands (blue arrows) at the membrane surface (right). Pro-92 and Arg-95 are expressed as blue circles, and Ala-112 as a green circle. Dashed lines indicate hydrogen bonds. Reorientation of the amido group of Arg-95 is shown as an open red arrow. The newly formed hydrogen bond between Arg-95 and Ala-112 is shown as a red dotted line.
solid state NMR spectroscopy is a powerful tool for obtaining structural information about peripheral membrane proteins at the membrane surface under conditions mimicking physiological conditions.

REFERENCES

1. Mayer, B. J., Ren, R., Clark, K. L., and Baltimore, D. (1993) Cell 73, 629–630
2. Haslam, R. J., Knade, H. B., and Hemmings, B. A. (1993) Nature 363, 309–310
3. Bottomley, M. J., Salim, K., and Panayotou, G. (1998) Biochim. Biophys. Acta 1436, 165–183
4. Hirata, M., Kanematsu, T., Takeuchi, H., and Yagisawa, H. (1998) Jpn. J. Pharmacol. 76, 255–263
5. Lemmon, M. A., and Ferguson, K. M. (1998) Curr. Top. Microbiol. Immunol. 228, 39–74
6. Lemmon, M. A., and Ferguson, K. M. (2000) Biochem. J. 350, 1–18
7. Cifuentes, M. E., Honkanen, L., and Rebecchi, M. J. (1993) J. Biol. Chem. 268, 11586–11593
8. Ramirez, F., and Jain, M. K. (1991) Proc. Struct. Funct. Genet. 9, 229–239
9. Rebecchi, M. J., Peterson, A., and McLaughlin, S. (1992) Biochemistry 31, 12742–12747
10. Lemmon, M. A., Ferguson, K. M., O’Brien, R., Sigler, P. B., and Schlessinger, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10472–10476
11. Ferguson, K. M., Lemmon, M. A., Schlessinger, J., and Sigler, P. B. (1995) Cell 83, 1037–1046
12. Yagisawa, H., Sakuma, K., Paterson, H. F., Cheung, R., Allen, V., Hirata, H., Watanabe, Y., Hirata, M., Williams, R. L., and Katan, M. (1998) J. Biol. Chem. 273, 417–424
13. Baek, K. J., Kang, S., Damron, D., and Im, M. (2001) J. Biol. Chem. 276, 5591–5597
14. Kang, S. K., Kim, D. K., Damron, D. S., Baek, K. J., and Im, M. J. (2002) Biochem. Biophys. Res. Commun. 293, 383–390
15. Tuzi, S., Hasegawa, J., Kawaminami, R., Naito, A., and Saito, H. (2001) Biophys. J. 81, 425–434
16. Saito, H., Tuzi, S., Yamaguchi, S., Tanio, M., and Naito, A. (2000) Biochim. Biophys. Acta 1460, 39–48
17. Tanio, M., Tuzi, S., Yamaguchi, S., Kawaminami, R., Naito, A., Needleman, R., Lanyi, J. K., and Saito, H. (1999) Biophys. J. 77, 1577–1584
18. Tanio, M., Inoue, S., Yokota, K., Seki, T., Tuzi, S., Needleman, R., Lanyi, J. K., Naito, A., and Saito, H. (1999) Biophys. J. 77, 431–442
19. Tuzi, S., Yamaguchi, S., Tanio, M., Konishi, H., Inoue, S., Naito, A., Needleman, R., Lanyi, J. K., and Saito, H. (1999) Biophys. J. 76, 1523–1531
20. Yagisawa, H., Hirata, M., Kanematsu, T., Watanabe, Y., Ozaki, S., Sakuma, K., Tanaka, H., Yabuta, N., Kamata, H., and Hirata, H. (1994) J. Biol. Chem. 269, 20179–20188
21. Saito, H. (1986) Magn. Reson. Chem. 24, 835–852
22. Saito, H., and Ando, I. (1989) Annu. Rep. NMR Spectrosc. 21, 209–290
23. Saito, H., Tuzi, S., and Naito, A. (1998) Annu. Rep. NMR Spectrosc. 36, 79–121
24. Tuzi, S., Naito, A., and Saito, H. (1994) Biochemistry 33, 15046–15052
25. Rothwell, W. P., and Waugh, J. S. (1981) J. Chem. Phys. 75, 2721–2722
26. Hristova, K., Wimley, W. C., Mishra, V. K., Anantharamiah, G. M., Segrest, J. P., and White, S. H. (1999) J. Mol. Biol. 290, 99–117
27. White, S. H., Ladokhin, A. S., Jayasinghe, S., and Hristova, K. (2001) J. Biol. Chem. 276, 32395–32398
Structure and Dynamics of the Phospholipase C-δ1 Pleckstrin Homology Domain Located at the Lipid Bilayer Surface
Satoru Tuzi, Naoko Uekama, Masashi Okada, Satoru Yamaguchi, Hazime Saitô and Hitoshi Yagisawa

J. Biol. Chem. 2003, 278:28019-28025. doi: 10.1074/jbc.M300101200 originally published online May 7, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300101200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 25 references, 6 of which can be accessed free at http://www.jbc.org/content/278/30/28019.full.html#ref-list-1