Title
Phosphoinositide specificity of and mechanism of lipid domain formation by annexin A2-p11 heterotetramer.

Permalink
https://escholarship.org/uc/item/1kg5g2k1

Journal
The Journal of biological chemistry, 280(52)

ISSN
0021-9258

Authors
Gokhale, Nikhil A
Abraham, Alexandra
Digman, Michelle A
et al.

Publication Date
2005-12-01

DOI
10.1074/jbc.m508129200

License
https://creativecommons.org/licenses/by/4.0/ 4.0

Peer reviewed
Phosphoinositide Specificity of and Mechanism of Lipid Domain Formation by Annexin A2-p11 Heterotetramer*

Received for publication, July 25, 2005, and in revised form, September 29, 2005 Published, JBC Papers in Press, October 17, 2005, DOI 10.1074/jbc.M508129200

Nikhil A. Gokhale, Alexandra Abraham, Michelle A. Digman, Enrico Gratton, and Wonhwa Cho

From the Department of Chemistry, University of Illinois, Chicago, Illinois 60607-7061 and the Department of Physics, University of Illinois, Urbana, Illinois 61801-3080

Annexin A2 is a phospholipid-binding protein that forms a heterotetramer (annexin II-p11 heterotetramer; A2t) with p11 (S100A10). It has been reported that annexin A2 is involved in binding to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) and in inducing membrane microdomain formation. To understand the mechanisms underlying these findings, we determined the membrane binding properties of annexin A2 wild type and mutants both as monomer and as A2t. Our results from surface plasmon resonance analysis showed that A2t and annexin A2 have modest selectivity for PtdIns(4,5)P₂ over other phosphoinositides, which is conferred by conserved basic residues, including Lys279 and Lys281, on the convex surface of annexin A2. Fluorescence microscopy measurements using giant unilamellar vesicles showed that A2t of wild type, but not (K279A)_2-(p11)_2 or (K281A)_2-(p11)_2, specifically induced the formation of 1-μm-sized PtdIns(4,5)P₂ clusters, which were stabilized by cholesterol. Collectively, these studies elucidate the structural determinant of the PtdIns(4,5)P₂ selectivity of A2t and suggest that A2t may be involved in the regulation of PtdIns(4,5)P₂ clustering in the cell.

Annexins are a family of peripheral proteins that bind anionic phospholipids in a Ca²⁺-dependent manner (1–5). Structures and in vitro functions of annexins have been well characterized. Annexins have a variable N-terminal region and a conserved C-terminal core that is composed of four (eight in case of annexin A6) α-helical annexin folds (2). The C-terminal core is the Ca²⁺-dependent membrane-binding module that contains multiple Ca²⁺-binding sites on its convex membrane-binding surface (2). The N-terminal region of annexins is attached to the concave side of the C-terminal core and thought to be involved in interactions with other proteins and post-translational modifications (3–5). In addition to their membrane-binding activities, annexins have been reported to have other in vitro activities, including membrane aggregation and lateral aggregation on the membrane surface (6). Despite the wealth of structural and functional information on annexins, their physiological functions are only beginning to emerge with recent genetic and cell studies (3–5).

Annexin A2 is an abundant cellular protein that has been implicated in numerous physiological processes (3–5, 7). Annexin A2 interacts with an EF-hand protein p11 (also known as S100A11) with high affinity via its N-terminal region, forming a symmetric heterotetramer, (annexin A2)₂-(p11)₂ (A2t)² (8, 9). Annexin A2 has been reported to exist either as a monomer or A2t in mammalian cells (3, 10–12). Annexin A2 and A2t have been shown to have high vesicle aggregating activity (13) and form a monolayer of protein clusters when bound to the lipid bilayer with anionic phospholipids accumulating underneath the protein clusters (14). Mounting evidence indicates that annexin A2 and A2t are involved in organizing cholesterol-rich lipid rafts (15, 16) and linking them to cytoskeletal proteins (17–20). It has been also reported that annexin A2 (21, 22) and A2t (22) bind to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) with high specificity and affinity and that this activity is linked to the organization of actin at membrane sites that are enriched in PtdIns(4,5)P₂. Together with previous reports showing that annexin A2 binds cholesterol-containing membranes (23, 24) and PtdIns(4,5)P₂ is localized in cholesterol-rich lipid rafts in the plasma membrane (25–28), these results suggest that annexin A2 plays a role in regulating the formation of PtdIns(4,5)P₂-rich lipid rafts or lipid raft-like structures. However, it is not known whether the annexin A2 dynamically controls the organization of these structures or it passively binds to the PtdIns(4,5)P₂-rich regions. Furthermore, the affinity and specificity of annexin A2 and A2t for PtdIns(4,5)P₂ have not been quantitatively determined, which makes it difficult to assess their capability to compete with other PtdIns(4,5)P₂-binding proteins under physiological conditions.

In this study, we systematically and quantitatively determined the phosphoinositide binding specificity and affinity of annexin A2 and A2t by surface plasmon resonance (SPR) analysis and identified the structural determinant of its phosphoinositide specificity. We also investigated the formation of PtdIns(4,5)P₂-rich membrane domains on giant unilamellar vesicles (GUV) induced by A2t and mutants under various conditions. Our study provides new insight into the mechanism by which A2t mediates the organization of PtdIns(4,5)P₂-rich membrane domains.

EXPERIMENTAL PROCEDURES

Materials—1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoinositol (POPI), and cholesterol were all purchased from Avanti Polar Lipids, Inc. (Alabaster, Alabama). For the phosphoinositide binding studies, PtdIns(4,5)P₂ was synthesized by reaction of PtdIns(3,4)P₂ with DTT. PtdIns(4,5)P₂ contains trace amounts of PtdIns(3)P and PtdIns(3,4,5)P₃. For the surface plasmon resonance experiments, PtdIns(4,5)P₂ was purified by reverse-phase HPLC (MDS Analytical Technologies, Sunnyvale, CA). Annexins A2 and A2t were prepared as described previously (22).

2 The abbreviations used are: A2t, annexin II-p11 heterotetramer; CHAPS, 3-(3-Cholamidopropyl)dimethylammonio)-1-propanesulfonic acid; GUV, giant unilamellar vesicle(s); LUV, large unilamellar vesicle(s); PH, pleckstrin homology; PLC, phospholipase C; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; POPI, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoinositol; POPS, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine; PtdIns(3)P, phosphatidylinositol 3-phosphate; PtdIns(4)P, phosphatidylinositol 4-phosphate; PtdIns(5)P, phosphatidylinositol 5-phosphate; PtdIns(3,4)P₂, phosphatidylinositol 3,4-bisphosphate; PtdIns(3,5)P₂, phosphatidylinositol 3,5-bisphosphate; PtdIns(3,4,5)P₃, phosphatidylinositol 3,4,5-trisphosphate; PtdIns(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; SPR, surface plasmon resonance; MARCKS, myristoylated alanine-rich C kinase substrate.
PtdIns(4,5)P₂ Clustering by Annexin A2

AL). 1,2-Dipalmitoyl derivatives of phosphatidylinositol 3-phosphate (PtdIns(3)P), phosphatidylinositol 4-phosphate (PtdIns(4)P), phosphatidylinositol 5-phosphate (PtdIns(5)P), phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P₂), phosphatidylinositol-3,5-bisphosphate (PtdIns(3,5)P₃), PtdIns(4,5)P₂, and phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P₃) were generous gifts from Dr. Karol Bruzik. The concentrations of the phospholipids were determined by a modified Bartlett analysis (29). 6-Dodecanoyl-2-dimethylaminonaphthalene (LAURDAN), BODIPY FL C₆,C₆-phosphatidylinositol 4,5-diphosphate (BODIPY-5PtdIns(4,5)P₂), fluorescein 5-isothiocyanate (“Isomer I”), and Texas Red™ C₂-maleimide were all purchased from Invitrogen. Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). CHAPS and octyl glucoside were purchased from Sigma and Fisher, respectively. The protease inhibitors, pepstatin, leupeptin, and aprotinin and the protease inhibitor mixture tablets were from Roche Applied Science. The Pioneer L1 sensor chip was purchased from Biacore AB (Piscataway, NJ).

Vector Construction and Mutagenesis—The cDNA of full-length human annexin A2, which was a generous gift from Dr. Volker Gerke, was subcloned into the vector pET-21a(+) (Novagen, Madison, WI), between the restriction sites BamHI and XhoI. A stop codon was introduced just before the restriction site XhoI in order to exclude the C-terminal hexahistidine tag from the sequence during protein expression. The cDNA of p11 (a generous gift from Dr. James Seilhamer) was also subcloned into pET-21a(+) in a similar fashion. K279A and K281A mutants of annexin A2 were generated by the overlap extension polymerase chain reaction mutagenesis. The vector pGEX-4T-1 (Novagen, Madison, WI), which has an N-terminal glutathione S-transferase tag and a thrombin cleavage site was used for subcloning the cDNA of the phospholipase C81 (PLC81) pleckstrin homology (PH) domain between the restriction sites BamHI and XhoI. All of the above constructs were transformed into DH5α cells for plasmid isolation, and their DNA sequences were verified. Subsequently, these plasmids were transformed into BL21(DE3) cells for protein expression.

Protein Expression and Purification—One liter of sterile Luria broth medium containing 100 µg/ml ampicillin was inoculated with BL21(DE3) cells harboring each construct and grown at 37 °C until the optical density at 600 nm reached 0.6. Protein expression was then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside (Research Products, Mount Prospect, IL). After 8 h of incubation at 37 °C, cells were harvested by centrifugation (5,000 g for 10 min at 4 °C). The resulting pellet was resuspended in 20 ml of precooled lysis buffer, containing 100 mM Tris- HCl (pH 7.5), 200 mM NaCl, 2 mM dithiothreitol, 1 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, 5 µg/ml pepstatin, 5 µg/ml aprotinin, a protease inhibitor tablet, and 0.1% Triton X-100. This suspension was sonicated for 6 min (30 s of sonication followed by 30 s of cooling on ice) and then centrifuged for 1 h (40,000 × g at 4 °C). The supernatant was treated with 50% (NH₄)₂SO₄ (final concentration) for 45 min and centrifuged at 40,000 × g for 20 min to remove insoluble proteins. This supernatant was then applied to an 80-ml butyl-Sepharose column (Amersham Biosciences) pre-equilibrated with 50% (NH₄)₂SO₄ in the lysis buffer. Annexin A2 (or a mutant) was eluted with a linear gradient of (NH₄)₂SO₄ from 50 to 0% in the lysis buffer. The eluted samples were dialyzed against 10 mM HEPES buffer, pH 7.5, containing 50 mM NaCl, 1 mM EGTA, 1 mM dithiothreitol, and a 2–5-fold molar excess of p11. These samples were then loaded onto a 5-ml Heparin HP HiTrap™ column (Amersham Biosciences) equilibrated with the same buffer without p11. The column was washed with 100 ml of the same buffer to get rid of excess p11, and the heterotetramer was eluted with 50 ml of elution buffer containing 30 mM HEPES, pH 7.5, 160 mM NaCl, 10 mM EGTA, and 1 mM dithiothreitol. The heterotetrameric nature of A2t was confirmed by the polyacrylamide gel electrophoresis performed under nondenaturing conditions (i.e. in the absence of SDS and dithiothreitol) using a 16% polyacrylamide gel (see Fig. 1). For the expression of the PLC81 PH domain, 1 liter of Luria broth containing 100 µg/ml ampicillin was inoculated with BL21(DE3) cells harboring the PH domain construct and grown at 37 °C until the optical density at 600 nm reached 0.4. Protein expression was induced by the addition of 50 µg of isopropyl-1-thio-β-D-galactopyranoside, and cells were harvested by centrifugation (5,000 × g for 10 min at 4 °C) after a 12-h incubation at 25 °C. The resulting pellet was resuspended in 10 ml of 30 mM HEPES buffer, pH 7.5, containing 160 mM NaCl, 50 µM phenylmethylsulfonyl fluoride, and 0.1% Triton X-100. This solution was sonicated for 6 min (30 s of sonication followed by 30 s of cooling on ice) and then centrifuged for 1 h (40,000 × g at 4 °C). After filtering the supernatant into a 50-ml Falcon tube, 500 µl of the glutathione S-transferase-Tag™ resin (Novagen, Madison, WI) were added. After incubating this mixture on ice for 45 min with mild shaking at 80 rpm, it was poured onto a column prewashed with 50 ml of 30 mM HEPES buffer, pH 7.5, containing 160 mM NaCl. After washing the nonspecifically bound protein with 50 ml of 30 mM HEPES buffer, pH 7.5, containing 160 mM NaCl, 1 ml of the same buffer containing 4 units of thrombin was added in order to cleave the glutathione S-transferase tag, and the column was then sealed for a 6-h incubation at 25 °C. The protein was then eluted in five fractions using 500 µl of 30 mM HEPES buffer, pH 7.5, containing 160 mM NaCl. The protein purity was checked on a 16% polyacrylamide gel, and all of the protein samples were subsequently concentrated. The protein concentrations were determined by the bicinchoninic acid method (Pierce).

Chemical Labeling of Proteins—The purified p11 (2 mg/ml) was treated with a 10-fold molar excess of Texas Red™ C₂-maleimide for 2 h at room temperature in 30 mM HEPES buffer, pH 7.5, which was treated with a 10-fold molar excess of Texas Red™ C₂-maleimide for 2 h at room temperature in 30 mM HEPES buffer, pH 7.5, which was
purged with a stream of nitrogen gas before use to remove oxygen. The labeling reaction was subsequently quenched by adding an excess amount of 2-mercaptoethanol, and the labeled protein was separated from the reagents using a Sephadex G25 column (Sigma) eluted with 30 mM HEPES buffer, pH 7.5, containing 50 mM NaCl. The fractions corresponding to the protein peak were pooled and dialyzed against 30 mM HEPES buffer, pH 7.5, containing 50 mM NaCl and 6 mM CaCl2 for 24 h at 4 °C. The labeling efficiency of p11 was estimated using the equation, mol of dye/mol of protein = (absorbance of the labeled protein at 582 nm)/(molar absorptivity of Texas Red at 582 nm (112,000 M−1 cm−1) × (protein concentration)). Under our labeling conditions, ~0.7 mol of Texas Red was incorporated per mol of p11. The labeled p11 was then incubated on ice with purified annexin A2 (wild type or mutants) for 2 h, and the labeled heterotetramer was purified using a HiTrap™ Heparin HP column as described above. The PLCβ1 PH domain was labeled with a 10-fold molar excess of fluorescein-5-isothiocyanate for 2 h at room temperature, and the labeled protein was separated using a Sephadex G25 column eluted with 30 mM HEPES buffer, pH 7.5, containing 160 mM NaCl.

**SPR Measurements**—All SPR equilibrium binding measurements were carried out at 23 °C as described (30, 31). The sensor chip Pioneer L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32). Typically, after washing the sensor chip surface, 90 L1 (Biacore) was coated with vesicles according to a protocol described previously (32).

**RESULTS**

**Phosphoinositide Specificity of Annexin A2 and A2t**—It has been recently reported that annexin A2 and A2t have high specificity for PtdIns(4,5)P2 (21, 22); however, this putative PtdIns(4,5)P2 specificity has not been quantitatively measured. We therefore prepared mixed vesicles of POPC/POPE containing 3 mol % of each of seven phosphoinositides and quantitatively determined by SPR analysis the affinity of annexin A2 and A2t for these vesicles coated onto the sensor chip. Annexin A2 and p11 have been shown to form a heterotetramer with high affinity (8, 9). Fig. 1 indicates that A2t exists as a heterotetramer under our experimental conditions. Representative sensorgrams for A2t and vesicles and a binding isotherm generated from the sensorgrams are shown in Fig. 2. The SPR method not only allows sensitive and quantitative determination of KD values (32, 35) but also circumvents the vesicle aggregation during binding measurements, because it uses vesicles immobilized on the sensor chip. This is important, because in the vesicle pelleting assay, the charge and size of vesicles can significantly affect the pelleting efficiency, which in turn complicates the interpretation of binding data (35). For the first set of measurements, a relatively high Ca2+ concentration (0.1 mM) was employed to ensure that the proteins show detectable affinities for all phosphoinositide-containing vesicles.
under the same conditions. The control surface was coated with POPC/POPE (80:20) vesicles, because neither annexin A2 nor A2t showed detectable affinity for these zwitterionic vesicles.

As summarized in TABLE ONE, A2t showed relatively high affinity (i.e. \( K_d = 33 \text{ nM} \)) for POPC/POPE/PtdIns(4,5)P_2 (77:20:3) vesicles, which was >10-fold higher than that for POPI- or POPS-containing vesicles. Also, this affinity is comparable with those of epsin 1 ENTH domain (36) and PLC\(\gamma\) PH domain (see TABLE ONE) for the same vesicles. However, annexin A2 monomer had >10-fold lower affinity for POPC/POPE/PtdIns(4,5)P_2 (77:20:3) vesicles than A2t under the same conditions. The difference was even bigger at lower Ca\(^{2+}\) concentrations. For instance, at 50 \( \mu \text{M Ca}^{2+}\), A2t had a \( K_d \) value of \( \sim 100 \text{ nM} \), whereas annexin A2 monomer showed no detectable affinity, suggesting that PtdIns(4,5)P_2-dependent membrane binding of annexin A2 monomer is physiologically insignificant. We therefore focused our measurements on A2t hereafter.

When compared among phosphoinositides, A2t showed only modest selectivity for PtdIns(4,5)P_2 over other phosphoinositides (i.e. its affinity for PtdIns(3,5)P_2-containing vesicles was less than 2-fold higher than that for vesicles containing PtdIns(3,4)P_2, PtdIns(3,5)P_2, and PtdIns(3,4,5)P_3, respectively, and less than 4-fold higher than that for vesicles containing each monophosphorylated phosphoinositide). Under the same conditions, the annexin A2 monomer also showed modest selectivity for PtdIns(4,5)P_2 over PtdIns(3)P, whereas p11 exhibited no appreciable phosphoinositide selectivity (see TABLE ONE). To preclude the possibility that this low PtdIns(4,5)P_2 selectivity is due to our experimental conditions employing vesicles containing 3 mol % phosphoinositide, we also determined the affinity of A2t for vesicles containing 1 mol % phosphoinositide. Comparison of \( K_d \) (equal to 96.8 \( \pm \) 6.7 nM) for POPC/POPE/PtdIns(4,5)P_2 (79:20:1) vesicles and \( K_d \) (equal to 156.6 \( \pm \) 7.0 nM) for POPC/POPE/PtdIns(3,4)P_2 (79:20:1) vesicles showed that selectivity of A2t for PtdIns(4,5)P_2 over PtdIns(3,4)P_2 remained essentially the same regardless of the concentration of phosphoinositide in the vesicles (see also TABLE ONE). Together, these data show that, unlike in previous reports (21, 22), neither annexin A2 monomer nor A2t has high specificity for PtdIns(4,5)P_2. However, the finding that A2t prefers PtdIns(4,5)P_2 to more anionic PtdIns(3,4,5)P_3 indicates that the observed PtdIns(4,5)P_2 selectivity of A2t does not simply derive from nonspecific electrostatic interactions.

This in turn suggests that A2t has a defined, albeit not optimized, PtdIns(4,5)P_2 binding site that is located not in p11 but in annexin A2.

The Effect of Other Lipids on Membrane Affinity of A2t—It has been reported that annexin A2 is involved in the organization of lipid rafts in the plasma membrane of mammalian cells (15, 16). To see if A2t has the physical properties that are consistent with this reported activity, we further investigated the membrane binding properties of A2t. First, we measured the affinities of A2t for the vesicles whose lipid headgroup compositions recapitulate those of mammalian cell membranes (see the footnotes to TABLE TWO) to assess the differential affinities of A2t for various cell membranes. This approach has been successfully applied to determine and account for the cellular targeting specificity of various membrane targeting domains and peripheral proteins (37, 38). For these measurements, we employed 5 \( \mu \text{M Ca}^{2+}\) to simulate the cellular environment as much as possible. Lower Ca\(^{2+}\) concentrations could not be used, because SPR signals were too small to analyze under such conditions.

As shown in TABLE TWO, A2t showed considerable affinity for the mimetic of inner plasma membrane at 5 \( \mu \text{M Ca}^{2+}\), while showing no detectable affinity for the mimetics of other cellular membranes. It should be noted that A2t exhibited no detectable binding to POPC/POPE/PtdIns(4,5)P_2 (77:20:3) vesicles at 5 \( \mu \text{M Ca}^{2+}\). Therefore, A2t has a preference for the lipid composition of the inner plasma membrane, suggesting that the plasma membrane is a main site for A2t actions. This preference became more pronounced when 3 mol % PtdIns(4,5)P_2 was incorporated into the plasma membrane mimetic. The \( K_d \) value for this membrane is 27 nM even at 5 \( \mu \text{M Ca}^{2+}\). Among various cellular membranes, the inner plasma membrane is known to contain the highest content of anionic lipids and cholesterol (see the footnotes to TABLE TWO), both of which have been shown to enhance the membrane affinity of annexin A2. Therefore, A2t showed no detectable affinity for this membrane even in the presence of 3 mol % PtdIns(4,5)P_2, demonstrating the importance of cholesterol in membrane binding of A2t. Interestingly, A2t did not show any binding to POPC/POPE/cholesterol (55:20:25) vesicles even at 1 mM Ca\(^{2+}\) (data not shown), indicating that A2t does not have affinity for cholesterol...
TABLE TWO

| Lipid composition (77:20:3) | Protein | $K_d$   | Increase in $K_d$ $^a$ |
|---------------------------|---------|---------|-----------------------|
| PM$^b$                    | Wild type | 282.7 ± 6.2 | 1.0 |
| EE$^c$                    | Wild type | ND$$^d$ |  |
| NM$^e$                    | Wild type | ND      |  |
| PM/PtdIns(4,5)P$_2$ $^f$  | Wild type | 27.4 ± 3.2 | 0.1$^e$ |
| PM/PtdIns(4,5)P$_2$-cholesterol$^g$ | Wild type | ND    |  |
| PM                         | K279A   | 499.7 ± 10.4 | 1.8 |
| PM                         | K281A   | 804.3 ± 45.9 | 2.8 |
| PM/PtdIns(4,5)P$_2$        | K279A   | 447.6 ± 37.0 | 1.6$^f$ |
| PM/PtdIns(4,5)P$_2$        | K281A   | 580.8 ± 17.9 | 2.1$^f$ |

$^a$ Fold increase in $K_d$ relative to the binding of wild type A2t to PM mimetic vesicles.
$^b$ PM, plasma membrane-mimicking POPC/POPE/POPS/POPI/cholesterol (12:35:22:9:22) vesicles (37, 66).
$^c$ EE, early endosomal membrane-mimicking POPC/POPE/POPS/POPI/PtdIns(3)P (62:20:15:3) vesicles (67).
$^d$ ND, not detectable.
$^e$ NM, nuclear membrane-mimicking POPC/POPE/POPS/POPI/cholesterol (61:21:4:7:7) vesicles (37, 66).
$^f$ PM/PtdIns(4,5)P$_2$ POPC/POPE/POPS/POPI/cholesterol/PtdIns(4,5)P$_2$ (12:35:22:9:22) vesicles.
$^g$ Note that K279A and K281A have 16- and 21-fold lower affinities, respectively, than wild type (shown in italic numbers) under the same conditions.
$^h$ POPC/POPE/POPS/PtdIns(4,5)P$_2$ (34:35:22:6) vesicles.

FIGURE 3. Sequence alignment of annexin A2 with actin- and PtdIns(4,5)P$_2$-binding proteins. Mammalian (human, mouse, and rat) A2 sequences were aligned with gelsolin, villin, GCAP39, MARCKS, and cortexillin I. The consensus nonapeptide motif is underlined, and two mutated cationic residues of annexin A2 (Lys279 and Lys281) are indicated by arrows.

PtdIns(4,5)P$_2$ Clustering and PtdIns(4,5)P$_2$-binding proteins are subdivided into two groups (38, 39). The first group contains a well defined structural module, such as PH domain, that specifically recognizes PtdIns(4,5)P$_2$ in a binding pocket, whereas the second group lacks a well defined binding pocket but utilizes a cluster of surface cationic residues to bind PtdIns(4,5)P$_2$. Annexin A2 should belong to the second group, since it does not have an established PtdIns(4,5)P$_2$-binding domain. To identify the annexin A2 residues involved in PtdIns(4,5)P$_2$ binding, we surveyed the PtdIns(4,5)P$_2$-binding motifs found in several actin-binding proteins belonging to the second group, including gelsolin, villin, gCAP39, MARCKS, and cortexillin I. These proteins have a consensus nonapeptide motif, (R/K)(R/K)(R/K) (see Fig. 3), which is directly involved in PtdIns(4,5)P$_2$ binding (40, 41). A multiple sequence alignment by ClustalW (42) (Fig. 3) revealed that annexin A2 also possesses near the C terminus a putative PtdIns(4,5)P$_2$-binding motif. Among several conserved basic residues in this region, Lys$^{279}$, Lys$^{281}$, and Arg$^{286}$ are located on the convex surface of the annexin core (see Fig. 4).

To see if these residues are involved in PtdIns(4,5)P$_2$ binding, we measured the effect of K279A and K281A mutations on the membrane affinity of A2t. R284A was not characterized, because this mutant was poorly expressed in *Escherichia coli*. When compared with A2t, (K279A)$_2$-(p11)$_2$ and (K281A)$_2$-(p11)$_2$ showed large 16- and 21-fold decreases, respectively, in the binding affinity for the plasma membrane mimetic containing 3 mol % PtdIns(4,5)P$_2$ at 5 μM Ca$^{2+}$ (see TABLE TWO). When the plasma membrane mimetic was used in the absence of PtdIns(4,5)P$_2$, however, (K279A)$_2$-(p11)$_2$ and (K281A)$_2$-(p11)$_2$ showed less than 3-fold lower affinity than A2t. These data indicate that Lys$^{279}$ and Lys$^{281}$ are involved in specific PtdIns(4,5)P$_2$ binding rather than nonspecific binding to the anionic membrane surface.

A2t-mediated Lipid Ordering and PtdIns(4,5)P$_2$ Clustering—Annexin A2 has been reported to induce lipid raft formation in mammalian cells (15, 16). However, these results are based mainly on detergent extraction and antibody-mediated visualization methods, in combination with cholesterol depletion, which provide only indirect evidence and may also cause artificial lipid clustering (43). To directly measure the effects of annexin A2 and A2t on the membrane organization, we employed a well defined *in vitro* system in which interactions of A2t and annexin A2 with the vesicles of different lipid composition and sizes could be directly monitored by various fluorescence techniques. We first measured spectrofluorometrically the annexin-mediated changes in lipid ordering in the plasma membrane mimetic vesicles (LUV of 100-nm diameter) containing 3 mol % PtdIns(4,5)P$_2$ and 1 mol % BODIPY-labeled PtdIns(4,5)P$_2$ and 0.1 mol % LAURDAN. It has been reported (33, 44) that an increase in the membrane order causes a blue shift in the fluorescence emission of the membrane-incorporated LAURDAN. As shown in Fig. 5A, A2t caused a significant blue shift of the LAURDAN emission spectra, suggesting that it induces membrane ordering. In contrast, (K279A)$_2$-(p11)$_2$ (Fig. 5B) and (K281A)$_2$-(p11)$_2$ (Fig. 5C) only slightly enhanced the emission intensity without a detectable spectral shift. Neither did annexin A2 cause a significant spectral shift (data not shown). Also, we measured the effect of cholesterol on the A2t-mediated membrane ordering. As shown in Fig. 5D, A2t did not cause the blue shift of LAURDAN fluorescence when cholesterol was removed from the above vesicles, suggesting that the presence of cholesterol is essential for A2t-mediated membrane ordering.

We then directly monitored the A2t-mediated clustering of PtdIns(4,5)P$_2$ using GUV of the plasma membrane mimetic containing 1 mol % BODIPY-labeled PtdIns(4,5)P$_2$ and 2 mol % unlabeled PtdIns(4,5)P$_2$. GUV (diameter > 10 μm) are an excellent model system for cell membranes that allow direct visualization of various membrane processes, including structural changes of membranes (45). For these
FIGURE 4. Location of PtdIns(4,5)P$_2$-binding residues in annexin A2. A, the structure of the C-terminal core of annexin A2 is shown in a ribbon diagram using atomic coordinates provided by Dr. Barbara Seaton. Three surface-exposed cationic residues in the PtdIns(4,5)P$_2$-binding loop, including Lys$^{279}$ and Lys$^{281}$, are shown in a yellow stick representation and labeled. Calcium ions are shown in magenta. B, the electrostatic potential surface of the same molecule. Red and blue qualitatively indicate negative and positive electrostatic potentials, respectively. The location of the PtdIns(4,5)P$_2$-binding loop is indicated by the arrow.

FIGURE 5. Effects of A2t and mutants on the LAURDAN fluorescence emission spectra. A, LAURDAN emission spectra in the plasma membrane-mimicking LUV containing 3 mol % PtdIns(4,5)P$_2$ and 0.1 mol % LAURDAN are blue-shifted after adding 40, 80, 120, 160, and 200 nM (from bottom to top in the spectra on the left) A2t. B, LAURDAN emission spectra in the same LUV after adding 0–1.0 µM (from bottom to top) K$_{279}$A$_2$-p11$_2$. C, LAURDAN emission spectra in the same LUV after adding 0–1.0 µM (from bottom to top) K$_{281}$A$_2$-p11$_2$. D, LAURDAN emission spectra after the addition of 0–1.0 µM (from bottom to top) of A2t to the same LUV minus cholesterol. 10 mM HEPES buffer, pH 7.5, with 0.16 M NaCl and 20 µM Ca$^{2+}$ was used for these measurements.
measurements, p11 was chemically labeled with Texas Red, and this fluorescently labeled p11 was incorporated into A2t, (K279A)₂-(p11)₂, and (K281A)₂-(p11)₂, respectively. A green fluorescence protein tag on either annexin A2 or p11 could not be used here, because it interfered with heterotetramer formation and membrane binding. p11 has a single surface-exposed Cys (Cys82) and another internal Cys (9). Treatment of p11 with Texas Red C₂-maleimide and partial purification yielded the labeled protein, which incorporated 0.7 mol of Texas Red/mol of p11, implying that only Cys82 is labeled. Most importantly, the labeled protein formed a heterotetramer with annexin A2 (see Fig. 1), and this labeled heterotetramer was indistinguishable from the unlabeled A2t with respect to the affinity for POPC/POPE/PtdIns(4,5)P₂ (77:20:3) vesicles (K_d ≈ 35 nM at 0.1 mM Ca²⁺).

In the absence of proteins, the BODIPY-labeled PtdIns(4,5)P₂ showed homogeneous distribution on the GUV surfaces (Fig. 6E, left). Ca²⁺ up to 1 mM had no effect on the distribution of PtdIns(4,5)P₂ (data not shown). The addition of 80 nM A2t to the GUV in the presence of 5 μM Ca²⁺, however, caused the formation of nearly 1-μm PtdIns(4,5)P₂ clusters (see Fig. 6, A and B, for clustering in two separate GUV). Most important, PtdIns(4,5)P₂ spots were invariably colocalized with A2t spots (Fig. 6, A and B), showing that PtdIns(4,5)P₂ clustering is directly linked to the PtdIns(4,5)P₂ binding of A2t. More than 80% of GUV used in this study showed the same trend. This notion was further supported by the finding that 300–800 nM (K279A)₂-(p11)₂ and (K281A)₂-(p11)₂ bound to GUV but did not form either PtdIns(4,5)P₂ clusters or protein aggregates under the same conditions (Fig. 6, C and D). Under the same conditions, the annexin A2 monomer (unlabeled) up to 1 μM did not cause PtdIns(4,5)P₂ clustering (Fig. 6E). Given that A2t has a greater tendency to laterally aggregate than annexin A2 at low Ca²⁺ concentrations (e.g., 5 μM), this indicates that A2t-mediated PtdIns(4,5)P₂ clustering derives not only from the capability of A2t to specifically bind PtdIns(4,5)P₂ but also from its ability to laterally aggregate on the vesicle surfaces.

To investigate the effect of cholesterol on the A2t-mediated PtdIns(4,5)P₂ clustering, we repeated the measurements using the same

**FIGURE 6.** A2t-induced PtdIns(4,5)P₂ clustering on GUV. A and B, BODIPY-PtdIns(4,5)P₂ (left) and Texas Red-labeled A2t (right) images of two separate GUV 5 min after adding 80 nM protein. C, BODIPY-PtdIns(4,5)P₂ (left) and Texas Red-labeled (K279A)₂-(p11)₂ (right) images 5 min after adding 800 nM protein. D, BODIPY-PtdIns(4,5)P₂ (left) and Texas Red-labeled (K281A)₂-(p11)₂ (right) images 5 min after adding 800 nM protein. E, BODIPY-PtdIns(4,5)P₂ images before (left) and 5 min after (right) adding 400 nM PLC₁₁ PH domain. F, BODIPY-PtdIns(4,5)P₂ images before (left) and 5 min after (right) adding 400 nM PLC₁₁ PH domain. G, BODIPY-PtdIns(4,5)P₂ (left) and Texas Red-labeled A2t (right) images after adding 200 nM A2t to the GUV of the plasma membrane mimetic containing 1 mol % BODIPY-PtdIns(4,5)P₂ and 2 mol % unlabeled PtdIns(4,5)P₂ in 10 mM HEPES buffer, pH 7.5, with 0.16M NaCl and 20 μM Ca²⁺. Images are recorded at 300 and 310 s to illustrate the transient nature of the clusters. H, 40 nM of the labeled A2t was incubated with the GUV of the plasma membrane mimetic containing 3 mol % unlabeled PtdIns(4,5)P₂ for 20 min, and 60 nM of the labeled PH domain was added. The left and middle panels show Texas Red-labeled A2t images before and 5 min after adding the PH domain, respectively, whereas the right panel illustrates the labeled PH domain image 5 min after the addition. The arrows indicate the locations of PtdIns(4,5)P₂/A2t clusters. The GUV of the plasma membrane mimetic containing 1 mol % BODIPY-PtdIns(4,5)P₂ and 2 mol % unlabeled PtdIns(4,5)P₂ in 10 mM HEPES buffer, pH 7.5, with 0.16M NaCl and 5 μM Ca²⁺ were used unless specified otherwise. Images were taken every 5 s.
PtdIns(4,5)P₂ Clustering by Annexin A2

GUV minus cholesterol. Since A2t has lower affinity for this membrane, Ca²⁺ concentration was raised to 20 μM (Kₐₕ = 150 nM for these vesicles at 20 μM Ca²⁺) to facilitate the membrane binding of A2t. Under these conditions, A2t-bound GUV formed protein and PtdIns(4,5)P₂ clusters on the membrane surfaces; however, the number of clusters was significantly reduced, and the clusters were only transient, as shown by the time lapse images in Fig. 6G. The clusters formed in the presence of cholesterol (see Fig. 6, A and B) lasted much longer than these transient clusters. This underscores the importance of cholesterol in the formation and stabilization of the PtdIns(4,5)P₂ clusters.

Last, we measured the effect of another PtdIns(4,5)P₂-binding protein, the PH domain of PLCδ1, to see whether the PtdIns(4,5)P₂ clustering is a generic property of any PtdIns(4,5)P₂-binding proteins or specific to A2t. We incrementally added the PLCδ1 PH domain to GUV of the plasma membrane mimetic containing 1 mol % BODIPY-labeled PtdIns(4,5)P₂ and 2 mol % unlabeled PtdIns(4,5)P₂. With the PH domain concentration up to 1 μM, under which condition the vesicle surfaces should be fully covered by the PH domain (note that Kₐₕ = 52 ns for the PH domain-PtdIns(4,5)P₂ vesicle binding; see TABLE ONE), no PtdIns(4,5)P₂ clustering was detected (Fig. 6F). We then chemically labeled the PLCδ1 PH domain with fluorescein to simultaneously monitor vesicle binding of A2t and the PH domain. For these measurements, BODIPY-labeled PtdIns(4,5)P₂ was not included in GUV to circumvent the spectral overlap with the fluorescein-labeled PH domain. When the fluorescein-labeled PH domain was added to the mixture after the Texas Red-labeled A2t was allowed to interact with the GUV of plasma membrane mimetic containing 3 mol % unlabeled PtdIns(4,5)P₂, in the presence of 5 μM Ca²⁺, the PH domain was able to rapidly bind to the GUV (Fig. 6H, right) without disrupting A2t clusters (Fig. 6H, left and middle). Notice that the locations of A2t clusters in the left and middle panels are different because they were monitored at different times, and PtdIns(4,5)P₂/A2t clusters diffused laterally on the membrane. This suggests that A2t-induced PtdIns(4,5)P₂ clusters are readily accessible to other PtdIns(4,5)P₂-binding proteins and may thus serve as sites for various PtdIns(4,5)P₂-mediated processes.

DISCUSSION

Although PtdIns(4,5)P₂ is a minor component of membrane lipids that is mainly found in the inner leaflet of plasma membrane, it plays important regulatory roles in diverse cellular processes, including actin polymerization (46, 47) and vesicle trafficking (48, 49), and also serves as the precursor for the generation of second messengers, including diacylglycerol, inositol (1,4,5)-trisphosphate (50), and PtdIns(3,4,5)P₃-dependent manner (21, 22), laterally aggregates on the membrane surface (14), interacts with F-actin (59), and is abundant in mammalian cells. Furthermore, recent reports have indicated the potential involvement of annexin A2 and A2t in regulation of membrane microdomain formation (15, 16). The present study quantitatively shows that A2t possesses all the biophysical properties that are necessary for its putative role in organizing PtdIns(4,5)P₂ clusters in the plasma membrane and also provides direct evidence for A2t-induced PtdIns(4,5)P₂ clusters in a well-defined model membrane system.

On the basis of qualitative lipid overlay assay and vesicle pelleting assay, Hayes et al. (21) recently reported that annexin A2 has high specificity and affinity for PtdIns(4,5)P₂. Rescher et al. (22) also reported that A2t as well as annexin A2 have high affinity and selectivity for PtdIns(4,5)P₂ in vesicle pelleting and lipid plate binding assays. In both studies, PtdIns(4,5)P₂ binding activity of annexin A2 and A2t was linked to the binding of these proteins to the sites of membrane-associated actin assembly in the cell. We reexamined the specificity and affinity of annexin A2 and A2t for PtdIns(4,5)P₂ by means of SPR analysis. Our SPR measurements confirm that annexin A2 and A2t bind PtdIns(4,5)P₂ and that A2t binds PtdIns(4,5)P₂-containing membranes with high affinity. The affinity of A2t for PtdIns(4,5)P₂-containing vesicles is comparable with that of the PLCδ1 PH domain and epsin ENTH domain (36). However, our measurements reveal that neither annexin A2 nor A2t has high specificity for PtdIns(4,5)P₂. The largest difference between PtdIns(4,5)P₂ and any phosphoinositide was less than 4-fold when they were incorporated in POPC/POPE/phosphoinositide (77: 20:3) vesicles. This discrepancy in PtdIns(4,5)P₂ specificity between the published data and ours should arise mainly from different methods used for binding measurements (see “Results”). A typical PtdIns(4,5)P₂-specific protein, such as PLCδ1 PH domain or epsin ENTH domain (36), shows much more pronounced selectivity for PtdIns(4,5)P₂ over other phosphoinositides. Thus, cellular specificity of A2t (or annexin A2) for PtdIns(4,5)P₂ would derive from the combination of modest PtdIns(4,5)P₂ selectivity of A2t and the relative abundance of PtdIns(4,5)P₂ over other phosphoinositides. In addition, the strong preference of A2t for the inner plasma membrane over other cellular membranes should help its specific interaction with PtdIns(4,5)P₂ enriched in the plasma membrane.

There is some confusion in the literature as to whether annexin A2 monomer or A2t is involved in vesicle trafficking and membrane microdomain organization (5). As reported previously (22), the modest PtdIns(4,5)P₂ selectivity of A2t derives not from p11 but from the annexin A2 molecule. However, the affinity of annexin A2 monomer for the PtdIns(4,5)P₂-containing membrane seems to be too low to be physiologically significant. It has been long known that A2t has lower Ca²⁺ requirement for phospholipid binding and granule aggregation than annexin A2 (60). However, the difference in membrane affinity between A2t and annexin A2 has not been quantified due in part to the complication associated with a conventional vesicle pelleting assay (see “Results”). Our SPR data show that A2t has at least 10 times higher affinity for PtdIns(4,5)P₂-containing vesicles than annexin A2 at 0.1 mM Ca²⁺ and that the difference is much bigger at lower Ca²⁺ concentrations. Presumably, A2t has much higher membrane affinity than annexin A2, because two membrane binding modules of A2t function either additively or synergistically, as proposed for the membrane bind-
ing of low affinity PH domains (61). Due to its low affinity, the annexin A2 monomer would not be able to compete with other PtdIns(4,5)P2-binding proteins for the same pools of PtdIns(4,5)P2 unless its membrane-bound proteins. These results thus indicate that A2t is the functional form of annexin A2 in the cell as far as the PtdIns(4,5)P2-dependent membrane binding is concerned.

This study also identifies the specific PtdIns(4,5)P2-binding residues in the annexin A2 molecule. Annexin A2 contains the basic "nonapeptide" motif that is found in many actin- and PtdIns(4,5)P2-binding proteins (40, 41). This motif is located in one of five Ca2+-binding loops. Since this loop protrudes in the middle of the convex surface (Fig. 4A) and has a highly positive electrostatic potential (Fig. 4B), it is expected to make direct contact with the anionic membrane. Among several cationic residues in this region, Lys279, Lys281, and Arg284 are surface-exposed, and at least two of them, Lys279 and Lys281, are shown to be directly involved in PtdIns(4,5)P2 binding. The K279A and K281A mutations reduce the affinity of A2t for the plasma membrane mimetic by about 20-fold. Thus, these residues do not represent the lipid raft-like structure. Since the resolution of light microscopy does not allow direct monitoring of lipid rafts, our study cannot distinguish whether A2t induces the formation of anionic lipid-rich microdomains or from randomly distributed lipids. In either case, the PtdIns(4,5)P2, binding activity of A2t appears to be essential for its effect on membrane organization. The ~1-µm size of PtdIns(4,5)P2 clusters formed on GUV is much larger than the putative size of cholesterol-rich lipid rafts (<250 nm), indicating that the observed PtdIns(4,5)P2 clusters do not represent the lipid raft-like structure. Since the resolution of light microscopy does not allow direct monitoring of lipid rafts, our study cannot distinguish whether A2t induces the formation of PtdIns(4,5)P2, clusters from preexisting cholesterol- and PtdIns(4,5)P2-rich lipid rafts or from randomly distributed lipids. In either case, the PtdIns(4,5)P2, clustering activity of A2t would seem to derive from its ability to laterally aggregate on the membrane surface, protein-bound PtdIns(4,5)P2 molecules also form patches, which is facilitated by the presence of cholesterol in the membrane. These A2t-induced PtdIns(4,5)P2 clusters are still accessible to other PtdIns(4,5)P2-binding proteins, as evidenced by undetected binding of the PLCG1 PH domain to the PtdIns(4,5)P2 clusters. In the cell, A2t is expected to bind the plasma membrane (or early endosomes under certain conditions) containing PtdIns(4,5)P2 and cause similar PtdIns(4,5)P2 clustering in a Ca2+-dependent manner, thereby generating spatially separated PtdIns(4,5)P2 pools. In this case, dual interactions of A2t with both the membrane and the cytoskeleton will severely limit the mobility of PtdIns(4,5)P2 clusters. Whether these PtdIns(4,5)P2 clusters correspond to lipid rafts or not, they may function as the sites for cell signaling, vesicle trafficking, or actin assembly. Obviously, further studies are necessary to elucidate if and how A2t mediates PtdIns(4,5)P2 clustering in the cell. This in vitro study provides important new structural and mechanistic information that will form the basis of such cell studies.

Acknowledgments—We thank Dr. Barbara Seaton for allowing use of the coordinates of annexin A2 prior to publication.

REFERENCES

1. Creutz, C. E. (1992) Science 258, 924–931
2. Swairjo, M. A., and Seaton, B. A. (1994) Annu. Rev. Biophys. Biomol. Struct. 23, 193–213
3. Gerke, V., and Moss, S. E. (2002) Physiol. Rev. 82, 331–371
4. Rescher, U., and Gerke, V. (2004) J. Cell Sci. 117, 2631–2639
5. Gerke, V., Creutz, C. E., and Moss, S. E. (2005) Nat. Rev. Mol. Cell Biol. 6, 449–461
6. Gerke, V., and Moss, S. E. (1997) Biochim. Biophys. Acta 1357, 129–154
7. Waisman, D. M. (1995) Mol. Cell. Biochem. 149, 301–322
8. Johnson, N., Marriot, G., and Weber, K. (1988) EMBO J. 7, 2435–2442
9. Rety, S., Sopkova, J., Renouard, M., Osterloh, D., Gerke, V., Tabaries, S., Russo-Marie, F., and Lewit-Bentley, A. (1999) Nat. Struct. Biol. 6, 89–95
10. Harder, T., and Gerke, V. (1994) Biochim. Biophys. Acta 1223, 375–382
11. Nilius, B., Gerke, V., Prener, J., Szucs, G., Heinke, S., Weber, K., and Droogmans, G. (1996) J. Biol. Chem. 271, 30631–30636
12. Konig, J., Prener, J., Nilius, B., and Gerke, V. (1998) J. Biol. Chem. 273, 19679–19684
13. Raynal, P., and Pollard, H. B. (1994) Biochim. Biophys. Acta 1179, 63–93
14. Menke, M., Ross, M., Gerke, V., and Steinem, C. (2004) ChemBiochem 5, 1003–1006
15. Oliferenko, S., Palha, K., Harder, T., Gerke, V., Schwarczer, C., Schwanz, H., Beug, H., Gunthert, U., and Huber, L. A. (1999) J. Cell Biol. 146, 843–854
16. Babichuk, E. B., and Draeger, A. (2000) J. Cell Biol. 150, 1113–1124
17. Harder, T., Kellner, R., Parrot, R. G., and Gruenberg, J. (1997) Mol. Biol. Cell 8, 533–545
18. Merrifield, C. J., Rescher, U., Almers, W., Prout, J., Gerke, V., Sechi, A. S., and Moss, S. E. (2001) Curr. Biol. 11, 1136–1141
19. Zobniak, N., Rescher, U., Laarmann, S., Michgohi, S., Schmidt, M. A., and Gerke, V. (2001) J. Cell Sci. 115, 91–98
20. Renaud, C., Gentil, B. J., Assand, N., Court, M., Garin, J., Delphin, C., and Baudier, J. (2004) J. Cell Biol. 164, 133–144
21. Hayes, M. J., Merrifield, C. J., Shao, D., Ayala-Sanmartin, J., Schoery, C. D., Levine, T. P., Prout, J., Curran, J., Bailly, M., and Moss, S. E. (2004) J. Biol. Chem. 279, 14157–14164
22. Rescher, U., Rubo, D., Lubwag, C., Zobniak, N., and Gerke, V. (2004) J. Cell Sci. 117, 3473–3480
23. Ayala-Sanmartin, J., Henry, J. P., and Pradel, L. A. (2001) Biochim. Biophys. Acta 1510, 18–28
24. Mayran, N., Parrot, R. G., and Gruenberg, J. (2003) EMBO J. 22, 3242–3253
PtdIns(4,5)P₂ Clustering by Annexin A2

25. Pike, L. J., and Casey, L. (1996) J. Biol. Chem. 271, 26453–26456
26. Pike, L. J., and Miller, J. M. (1998) J. Biol. Chem. 273, 22298–22304
27. Waugh, M. G., Lawson, D., Tan, S. K., and Hsuan, J. J. (1998) J. Biol. Chem. 273, 17111–17121
28. Waugh, M. G., Minogue, S., Anderson, J. S., dos Santos, M., and Hsuan, J. J. (2001) J. Biol. Chem. 276, 9102–9123
29. Stahelin, R. V., and Cho, W. V. (2001) Current Opin. Cell Biol. 13, 657–666
30. Malloy, N. J., Han, S., Smiley, K. L., and Grinstein, S. (2002) J. Biol. Chem. 277, 7535–7542
31. Brown, D. A., and London, E. (1998) Biochemistry 37, 1455–1472
32. Pinyopich, A., and Grinstein, S. (2000) Biochemistry 39, 3699–3705
33. Bos, J. L., and Grinstein, S. (2002) J. Biol. Chem. 277, 1037–1044
34. Rhee, S. G., and Grinstein, S. (1999) J. Biol. Chem. 274, 1455–1462
35. Terebiznik, M. R., Vieira, O. V., Marcus, S. L., Slade, A., Yip, C. M., Trimble, W. S., Meyer, T., Finlay, B. B., and Grinstein, S. (2002) Nat. Cell Biol. 4, 766–773
36. Rho, J. M., and Grinstein, S. (2003) J. Biol. Chem. 278, 36332–36337
37. Janmey, P. A., and Lindberg, U. (2004) Nat. Rev. Mol. Cell Biol. 5, 658–666
38. Botelho, R. J., Teruel, M., Dierckman, R., Anderson, R., Wells, A., York, J. D., Meyer, T., and Grinstein, S. (2000) J. Cell Biol. 151, 1353–1368
39. Terebiznik, M. R., Vieira, O. V., Marcus, S. L., Slade, A., Yip, C. M., Trimble, W. S., Meyer, T., Finlay, B. B., and Grinstein, S. (2002) Nat. Cell Biol. 4, 766–773
40. Watt, S. A., Kular, G., Fleming, I. N., Downes, C. P., and Lucocq, J. M. (2002) Biochem. J. 363, 657–666
41. Huang, S., Lifshitz, L. Patki-Kamath, V., Tuf, R., Fogarty, K., and Czech, M. P. (2004) Mol. Cell Biol. 24, 9102–9123
42. Lauz, T., Fukami, K., Thelen, M., Golub, T., Frey, D., and Caroni, P. (2000) J. Cell Biol. 149, 1455–1472
43. Filipenko, N. R., and Waisman, D. M. (2001) J. Biol. Chem. 276, 5310–5315
44. Drust, D. S., and Creutz, C. E. (1988) Nature 331, 88–91
45. Klein, D. E., Lee, A., Frank, D. W., Marks, M. S., and Lemmon, M. A. (1998) J. Biol. Chem. 273, 27725–27733
46. Ford, M. G., Pears, B. M., Higgins, M. K., Vallis, Y., Owen, D. J., Gibson, A., Hopkins, C. R., Evans, P. R., and McMahon, H. T. (2001) Science 291, 1051–1055
47. Ayalasamartin, J. (2001) Biochem. Biophys. Res. Commun. 283, 72–79
48. Ross, M., Gerke, V., and Steinem, C. (2003) Biochemistry 42, 3131–3141
49. Brown, D. A., and London, E. (1998) Annu. Rev. Cell Dev. Biol. 14, 111–136
50. McMurray, W. C. (1973) in Techniques of Lipidology, 3rd Ed., Elsevier, Amsterdam, The Netherlands
51. Kobayashi, T., Stang, E., Fang, K. S., de Moerloose, P., Parton, R. G., and Gruenberg, J. (1998) Nature 392, 193–197
Phosphoinositide Specificity of and Mechanism of Lipid Domain Formation by Annexin A2-p11 Heterotetramer
Nikhil A. Gokhale, Alexandra Abraham, Michelle A. Digman, Enrico Gratton and Wonhwa Cho

J. Biol. Chem. 2005, 280:42831-42840.
doi: 10.1074/jbc.M508129200 originally published online October 17, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M508129200

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 65 references, 34 of which can be accessed free at http://www.jbc.org/content/280/52/42831.full.html#ref-list-1