The C Terminus of Annexin II Mediates Binding to F-actin

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Nolan R. Filipenko and David M. Waisman‡

From the Cancer Biology Research Group, Departments of Biochemistry and of Molecular Biology and Oncology, University of Calgary, Calgary, Alberta T2N 4N1, Canada

Annexin II heterotetramer (AIIt) is a multifunctional Ca\textsuperscript{2+}-binding protein composed of two 11-kDa subunits and two annexin II subunits. The annexin II subunit contains the binding sites for anionic phospholipids, heparin, and F-actin, whereas the p11 subunit provides a regulatory function. The F-actin-binding site is presently unknown. In the present study we have utilized site-directed mutagenesis to create annexin II mutants with truncations in the C terminus of the molecule. Interestingly, a mutant annexin II lacking its C-terminal 16, 13, or 9 amino acids was unable to bind to F-actin but still retained its ability to interact with both anionic phospholipids and heparin. Recombinant AIIt, composed of wild-type p11 subunits and the mutant annexin II subunits, was also unable to bundle F-actin. This loss of F-actin bundling activity was directly attributable to the inability of mutant AIIt to bind F-actin. These results establish for the first time that the annexin II C-terminal amino acid residues, LLYLCCGDD, participate in F-actin binding.

The cortical region of eukaryotic cells is made up of the plasma membrane and the underlying protein meshwork of the cytoskeleton. The cortical cytoskeleton plays a role in phenomena such as cellular morphology, membrane domain specialization, and cell-cell and cell-substratum interactions. In addition, the cortical cytoskeleton is involved in signal transduction events that regulate membrane trafficking, cell migration, and growth regulation. A major portion of the cortical cytoskeleton is made up of actin filaments whose physiological state is governed by actin-binding proteins. These actin-binding proteins control actin filament polymerization, filament-filament interaction, and filament-plasma membrane interaction (reviewed in Ref. 8).

The proteins that link the actin cytoskeleton with the plasma membrane are particularly diverse in their actions. An example is the ezrin-radixin-moesin (ERM) family of proteins (9).

These proteins are known to be involved in cytoskeletal-membrane events such as cell adhesion (10–12), Rho- and Rac-mediated cell morphology (13–16) as well as Akt-mediated cell survival (17). Further discovery and characterization of cortical actin-binding proteins will be important in elucidating the in vivo roles that the eukaryotic cell cortex and its binding partners play within the cell.

Annexin II is a member of the annexin family of proteins that are well characterized by their ability to bind to acidic phospholipids in a Ca\textsuperscript{2+}-dependent manner (18). In addition to their phospholipid binding activity, most family members discovered to date bind F-actin in a Ca\textsuperscript{2+}-dependent manner (19). Annexin II is unique among the annexins in that it exists as both a monomer and a tetramer within the cell. Annexin II tetramer (AIIt) consists of two copies of annexin II bound to a dimer of p11, a member of the S-100 family of Ca\textsuperscript{2+}-binding proteins. Within the cell, it has been established that annexin II is localized throughout the cell, whereas AIIt is localized to the plasma membrane-actin cytoskeleton interface (20–23). It has been suggested that intracellular AIIt acts as a link between the cytoskeleton and the plasma membrane, although the physiological consequences of this proposed interaction are unknown (24).

Although both annexin II and AIIt bind F-actin in vitro, only AIIt bundles F-actin (25). The bundling of F-actin by AIIt was rapid and reversible (t\textsubscript{0.5} = 6 s) and of moderate affinity (apparent K\textsubscript{d} (AIIt) of 0.18 μM). This process was dependent on the presence of Ca\textsuperscript{2+}, with half-maximal values obtained near 2 μM Ca\textsuperscript{2+}. The region of annexin II that contributes to the bundling activity of AIIt bundling activity was a region of α-helix in the fourth domain of annexin II that exhibited homology to an actin-binding site of myosin (26). Although this region of annexin II was critical for the F-actin bundling activity of AIIt, it did not participate in F-actin binding. In contrast to the F-actin bundling domain of AIIt, the F-actin binding domain of annexin II has remained elusive.

Here we present evidence establishing that the C terminus of the annexin II subunit of AIIt comprises an F-actin binding domain. Interestingly, the last 9 amino acid residues of the C terminus of the annexin II subunit appear to be entirely responsible for the F-actin binding activity of AIIt.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis of Annexin II**—A bacterial expression vector (pAE4.91) containing the wild-type sequence for annexin II was mutagened using the QuikChange site-directed mutagenesis kit (Stratagene). Briefly, mutagenic primers were synthesized to introduce premature stop codons immediately following Ala\textsuperscript{329}, Asp\textsuperscript{325}, and Thr\textsuperscript{322} in the annexin II cDNA. The resultant plasmids encoded for a series of C-terminal annexin II subunit (Ser\textsuperscript{1}-Asp\textsuperscript{325}); CT\textsubscript{16} AIIt, recombinant AIIt composed of wild-type p11 subunit and mutant annexin II subunit (Ser\textsuperscript{1}-Thr\textsuperscript{322}); PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; K<sub>c</sub>, total ligand concentration for half-maximal response; MOPS, 4-morpholinepropanesulfonic acid.
terminally truncated annexin II proteins, which we have named CTΔ9, CTΔ13, and CTΔ16, respectively. These plasmids were then transformed into Escherichia coli BL21(DE3) and grown as previously described (27).

Purification of Wild-type and C-terminal Terminated Annexin II—After 4 h of induction with isopropyl-1-thio-β-D-galactopyranoside, bacteria were collected by low speed centrifugation. They were subsequently sonicated in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM DTT) containing protease inhibitors, and following centrifugation, the annexin II mutants were found to be insoluble, contrary to wild-type annexin II. The mutant annexin II proteins were solubilized using 6 μM guanidinium hydrochloride followed by dialysis against several changes of buffer A. After solubilization, the mutant annexin II proteins were purified in the same manner as wild-type annexin II via hydroxyapatite, heparin-Sepharose affinity, and gel permeation chromatography as reported previously (27). The elution profiles of the recombinant wild-type and mutant annexin II on hydroxyapatite, heparin affinity, and gel permeation chromatography were indistinguishable. In addition, the circular dichroism spectra of each of the proteins were very similar, indicating little secondary structure perturbation.

Purification of Recombinant p11—The production of the recombinant p11 construct and the subsequent protein purification scheme have been previously reported (27, 28). Briefly, bacteria were grown and induced with isopropyl-1-thio-β-D-galactopyranoside as described above. The resultant cell lysate supernatant was then applied to a Fast Q ion-exchange column, after which Fast S ion-exchange chromatography was performed on the unbound flowthrough fraction, followed by gel permeation chromatography.

Purification of Recombinant Annexin II Tetramer—Equimolar amounts of recombinant annexin II (WT, CTΔ9, CTΔ13, or CTΔ16) and recombinant p11 were incubated at 4 °C for 30 min and then subjected to gel permeation chromatography to separate the monomeric from the tetrameric forms of annexin II. The elution profiles of the recombinant WT and CTΔ9, CTΔ13, and CTΔ16 AIIt were identical, suggesting that a gross change in the conformation of the mutant AIIt had not occurred as a result of the truncations. Proteins were subsequently stored in buffer A at –80 °C until use.

Phospholipid Vesicle Aggregation Assay—In a 12- × 75-mm culture tube, 50 μl each of 20 mg/ml phosphatidylserine, phosphatidylethanolamine, and cholesterol (dissolved in chloroform) were shelled by N2 gas. The resultant residue of lipids was resuspended in 1 ml of phospholipid aggregation buffer (30 mM HEPES, pH 7.5, 50 mM KCl) and sonicated at 75 watts for three 10-s bursts with a Braun probe sonicator to generate phospholipid vesicles. This stock lipid solution, at a concentration of 1 mg/ml, was prepared fresh daily. Phospholipid vesicle aggregation was assayed in an ELx-808 spectrophotometric plate reader (Bio-Tek Instruments) at 450 nm in a final volume of 200 μl. The resultant cell lysate supernatant was then applied to a Fast Q ion-exchange column, after which Fast S ion-exchange chromatography was performed on the unbound flowthrough fraction, followed by gel permeation chromatography.

Phospholipid Vesicle Binding Assay—Phospholipid vesicle binding was performed as described above for vesicle aggregation. The reaction was initiated by the addition of varying amounts of annexin II and incubated at room temperature for 15 min. After the incubation period, the reaction mixture was centrifuged at 14,000 × g in a desktop centrifuge. The pelletted phospholipid vesicles were resuspended in 30 μl of SDS sample buffer and boiled for 3–5 min (29). The phospholipid vesicle pellet was then resolved by SDS-PAGE and stained with Coomassie Blue, and densitometric analysis of the annexin II band was performed using a Hewlett Packard ScanJet 4c flatbed scanner and ImageQuaNT software (Molecular Dynamics). The densitometric intensity of annexin II centrifuged in phospholipid aggregation buffer without phospholipid vesicles was subtracted from all readings. Unless otherwise stated, the results are expressed as the percentage maximum of phospholipid binding by either WT annexin II or AIIt.

F-actin Bundling Assay—F-actin binding was measured by the light scattering intensity perpendicular to the incident light in a PerkinElmer Life Sciences LS-50B luminescence spectrophotometer as described previously (25). The excitation and emission wavelengths were both set to 400 nm with slit widths of 10 nm and 5 nm, respectively. F-actin (0.60 μM) was incubated in bundling buffer (25 mM MOPS, pH 7.0, 0.5 mM DTT, 0.33 mM ATP, 500 μM CaCl2) to a final volume of 600 μl. F-actin binding was initiated by the addition of varying amounts of WT, CTΔ9, CTΔ13, or CTΔ16 AIIt. After a 15-min incubation at room temperature, light scattering intensity was recorded in at least triplicate. The light scattering intensity of F-actin alone in bundling buffer was subtracted from all readings.

F-actin Binding Assay—F-actin binding was performed as described above for bundling, except that the reaction volume was adjusted to 200 μl. After a 15-min incubation period, the sample was centrifuged at 400,000 × g for 30 min in a Beckman Optima TLX Ultracentrifuge. The pellet was solubilized at room temperature for 1 h in 25 μl of 100 mM KCl and SDS sample buffer, followed by boiling for 3–5 min. To quantify the protein remaining in the supernatant after centrifugation, the supernatants were concentrated using chloroform/methanol precipitation as described previously (31). The precipitation protein was treated with SDS sample buffer as described above for the pelleted fraction. The pellet fractions or the concentrated supernatant fractions were then resolved by SDS-PAGE, followed by densitometric analysis as described above for the phospholipid binding assay. The densitometric intensity of annexin II centrifuged in bundling buffer without F-actin was subtracted from all readings. The results, unless otherwise stated, are expressed as the percentage maximum of actin binding by either WT annexin II or AIIt.

Miscellaneous Techniques—Protein concentrations were determined using Coomassie Brilliant Blue, and standardizing the concentrations to BSA, as described by Bradford (32). All reagents used were of analytical grade purity. Data was analyzed using Sigma Plot (Jandel Scientific).

RESULTS
C-terminal Actin-binding Motifs—The C terminus of many actin-binding proteins contains the F-actin binding domain. Examples include the villin superfamily (33), the 34-kDa Dictyostelium discoideum bundling protein (34), vinculin (35), human erythroid dematin (36), and the ERM proteins (37, 38). Interestingly, many F-actin-binding proteins, such as the ERM family and AIIt, are thought to form a link between the actin cytoskeleton and the plasma membrane. Furthermore, the ERM proteins and AIIt form F-actin bundles as a consequence of their F-actin binding activity (18, 25, 26, 39–42). We therefore investigated the possibility that the F-actin-binding site of AIIt was also contained within its C terminus.

Initially, we constructed a mutant AIIt composed of native p11 and mutant annexin II subunits. The mutant annexin II subunit was constructed such that the 16 amino acids of the C terminus were truncated (CTΔ16 AIIt). As shown in Fig. 1, analysis of the F-actin bundling activity of CTΔ16 AIIt by light scattering revealed that the F-actin bundling activity of this mutant AIIt was severely diminished compared with WT AIIt. To confirm that the decrease in light scattering was due to a loss in the formation of supramolecular structures (bundles) of F-actin, we took advantage of the fact that F-actin does not sediment at 14,000 × g but that F-actin bundles can be harvested at this centrifugal force. The lack of bundling activity by the CTΔ16 AIIt was thus shown to be due to a reduced interaction between F-actin and the mutant AIIt (Fig. 1, inset). This suggested that the C-terminal region of annexin II was important in mediating the F-actin binding.

To further characterize this domain, we truncated the C terminus of annexin II by 9 (CTΔ9) and 13 (CTΔ13) amino acid residues and examined the F-actin binding activity of these proteins.

F-actin Binding by Mutant Annexin II—It has been previously shown that annexin II binds F-actin in a Ca2+-dependent manner in vitro (19, 42). We assessed the ability of the C-terminal truncated annexin II mutants (CTΔ9, CTΔ13, and CTΔ16) to bind to F-actin using high speed centrifugation (400,000 × g). Under these experimental conditions, F-actin-binding proteins will cosediment with F-actin. As shown in Fig. 2A, at submaximal annexin II concentrations the truncated proteins have decreased F-actin binding, together with a shift in the K0.5 (annexin II). Furthermore, addition of 1 mg/ml BSA did not affect this binding phenomenon (data not shown), sug-
A shift in the Ca$^{2+}$—
bind to phospholipid vesicles in a Ca$^{2+}$-dependent manner (Fig. 3A, inset). The Ca$^{2+}$-dependence of F-actin binding by WT, CT$\Delta$9, CT$\Delta$13, and CT$\Delta$16 annexin II that cosedimented with F-actin was 45, 50, and 60% that of WT annexin II, respectively.

We next measured the ability of both WT and mutant annexin II to interact with F-actin at different concentrations of Ca$^{2+}$. This was done to ensure that all F-actin binding at submaximal annexin II concentrations is not due to a shift in the Ca$^{2+}$-dependence of the F-actin-annexin II interaction. As shown in Fig. 2B, the mutant annexin II proteins showed approximately a 5-fold reduction in F-actin binding in the presence of 1 mM Ca$^{2+}$ compared with WT annexin II. Thus, the decrease in F-actin binding by mutant annexin II was not due to a decrease in the Ca$^{2+}$-dependence of F-actin binding.

Phospholipid Aggregation and Binding by Mutant Annexin II—The Ca$^{2+}$-dependent binding of annexin II to phospholipid liposomes is the most studied phenomenon of the protein (reviewed in Ref. 24). Fig. 3A is a comparison of the relative abilities of WT and mutant annexin II to aggregate phospholipid vesicles. In the presence of EGTA, none of the proteins were able to aggregate phospholipid vesicles. However, all proteins aggregated phospholipid liposomes equally in the presence of either 20 or 200 mM Ca$^{2+}$.

We next examined the ability of the mutant annexin II to bind to phospholipid vesicles in a Ca$^{2+}$-dependent manner (Fig. 3A, inset). The CT$\Delta$9, CT$\Delta$13, and CT$\Delta$16 annexin II, in a similar manner and capacity to WT annexin II, pelleted with the phospholipid vesicles in the presence of Ca$^{2+}$ (either 20 or 200 mM Ca$^{2+}$). In the absence of Ca$^{2+}$, neither WT annexin II nor mutant annexin II bound to the phospholipid vesicles.

The dose dependence of phospholipid vesicle binding by WT, CT$\Delta$9, CT$\Delta$13, and CT$\Delta$16 annexin II is shown in Fig. 3B. In the presence of Ca$^{2+}$, the WT, CT$\Delta$9, CT$\Delta$13, and CT$\Delta$16 annexin II all bound phospholipid vesicles with similar affinity and capacity. Thus, truncation of the C terminus of annexin II did not affect the phospholipid binding activity of the protein.

**F-actin Bundling and Binding by Mutant AII**—Although annexin II contains the phospholipid- and F-actin-binding sites, it is the tetrameric form of the protein that has been localized to the cortical actin cytoskeleton (20, 21, 23, 43, 44). It is known that the interaction of the annexin II subunits with the p11 subunits causes a change in the conformation of both proteins (45). Therefore, it is unclear whether the C terminus of the annexin II subunit within AII is equivalent in structure or function to that of annexin II monomer.

Upon Ca$^{2+}$-dependent binding to F-actin, AII is, but not an-
Annexin II, rapidly and reversibly forms anisotropic F-actin bundles (25). We therefore analyzed the ability of C-terminally truncated AIIt mutants to bundle actin filaments using light scattering. After a 15-min incubation period, the reaction mixtures were centrifuged at 14,000 × g and phospholipid vesicle-associated annexin II was resolved by SDS-PAGE. The amount of annexin bound was then quantitated by densitometric analysis of the Coomassie Blue-stained gel. The results are expressed as the percentage of maximal binding displayed by WT AIIt and representative of three independent experiments.

We next examined whether or not the lack of F-actin bundling activity displayed by the AIIt mutants was due to a decreased binding affinity for F-actin. As shown in Fig. 4B, the mutant forms of AIIt display only about 15–20% of the F-actin binding activity by light scattering (mean ± S.D., n = 5). B, concentration dependence of F-actin binding by WT, CTΔ9, CTΔ13, and CTΔ16 AIIt. F-actin (0.6 μM) was incubated at 20 °C in a buffer containing 25 mM MOPS, pH 7.0, 50 mM KCl, 0.33 mM ATP, 0.5 mM DTT, and 500 μM CaCl2 (600 μl of total volume). WT (filled circles), CTΔ9 (open circles), CTΔ13 (filled triangles), or CTΔ16 (open triangles) AIIt was added at the indicated concentrations to initiate the reaction. After a 10-min incubation period, the reactions were analyzed for F-actin binding activity by light scattering (mean ± S.D., n = 5). B, concentration dependence of F-actin binding by WT, CTΔ9, CTΔ13, and CTΔ16 AIIt. F-actin (0.6 μM) was incubated at 20 °C in a buffer containing 25 mM MOPS, pH 7.0, 50 mM KCl, 0.33 mM ATP, 0.5 mM DTT, and 500 μM CaCl2 (600 μl of total volume). WT (filled circles), CTΔ9 (open circles), CTΔ13 (filled triangles), or CTΔ16 (open triangles) AIIt were added at the indicated concentrations and the reaction mixture was incubated at 20 °C for 10 min. After the incubation period, the samples were centrifuged at 400,000 × g for 30 min and then resolved by SDS-PAGE. The AIIt bound to F-actin was quantitated by densitometric analysis of the Coomassie Blue-stained gel. Results are expressed as the percentage of maximal binding displayed by WT AIIt and representative of three independent experiments.

DISCUSSION

Annexin II tetramer (AIIt) is a multifunctional protein that interacts in a Ca2+–dependent manner with anionic phospholipids, F-actin, and heparin (18). Due to its ability to bind simultaneously to actin and acidic phospholipids, it is thought to act within cells as a membrane-cytoskeleton linking protein.
(24). It is well established that the annexin II subunit of the protein complex houses the binding sites for the aforementioned ligands, as well as the sites for Ca\(^{2+}\) coordination (18). However, the exact location of the F-actin-binding sites within the protein has remained elusive. The similarity in function between the ERM proteins and AIIt, and the presence of the F-actin binding domain within the C terminus of the ERM proteins, led us to suspect that the C-terminal region of the annexin II subunit of AIIt might be involved in F-actin binding. It is known that the N terminus of annexin II is responsible for binding of p11 (23, 46, 47), however, a role has not been attributed to the C-terminal region of the protein. Therefore, we have used site-directed mutagenesis to truncate the C terminus of annexin II to assess its possible function in F-actin binding.

We initially observed that truncation of the last 16 amino acid residues of the C terminus of the annexin II subunit of AIIt inhibited the F-actin bundling activity of the protein by about 90%. This led us to suspect that the C terminus was involved in the interaction with F-actin. We then examined the F-actin binding activity of the isolated annexin II subunit. Analysis of the F-actin binding properties of several C-terminal truncations of annexin II revealed that removal of 16 or even as few as 9 amino acid residues also inhibited the F-actin binding activity of the protein. Furthermore, the truncations in the C terminus of annexin II had no effect on the interaction of annexin II with phospholipid vesicles or heparin. The C-terminally truncated annexin II mutant proteins bound to and aggregated acidic phospholipid vesicles in a Ca\(^{2+}\)-dependent manner with a similar affinity and capacity as WT annexin II. Furthermore, mutant annexin II bound to a heparin-Sepharose affinity column in a similar manner as WT annexin II (data not shown). Finally, to determine whether the annexin II monomer behaved similarly to annexin II that was complexed with p11, we assessed the ability of the AIIt, composed of wild-type p11 subunits and C-terminally truncated annexin II subunits to bundle F-actin filaments. Clearly, AIIt composed of wild-type p11 subunits and C-terminally truncated annexin II subunits was unable to bundle F-actin. Further investigation revealed that the loss in F-actin bundling was due to a decrease in the binding of the mutant AIIt to F-actin.

Our results therefore identify for the first time that the C-terminal sequence of the annexin II subunit of AIIt, LLYLGCGGDD, contains an F-actin binding domain of AIIt. The localization of the F-actin binding domain to the C-terminal 9 amino acid residues of annexin II represents one of the smallest F-actin binding domains discovered to date. Thymosin \(\beta_4\) also contains a relatively small actin-binding site. In this protein, a region comprising the first 12 amino acid residues of the protein forms the actin-binding site (48, 49). However, thymosin \(\beta_4\) is a well-characterized G-actin-sequestering protein, with only a weak affinity for F-actin (50–52). Known F-actin binding domains, however, are quite variable in length, with no consensus sequence length among them. For example, the F-actin-binding sites of the ERM proteins vary from 22 to 34 amino acids (14, 38). As well, an F-actin binding domain recently localized in myosin light-chain kinase spans amino acid residues 2–42 of the protein (53). Even in light of the above examples, most F-actin-binding sites encompass substantially larger portions of the protein. For example, the F-actin-binding site of vinculin is 123 amino acid residues in length (35). In addition, the three identified F-actin-binding sites within the D. discoideum 34-kDa F-actin-bundling protein vary in length from 16 to 123 amino acid residues in length, although the 16-amino acid residue domain has only weak binding to F-actin (34).

Although the overall length of the F-actin-binding site of annexin II is relatively small compared with known sites, other requirements for F-actin binding within this site appear to be met. For example, the structural requirements of prototypical F-actin-binding sites are fulfilled when discussing the C terminus of annexin II. It is known that the majority of F-actin-binding sites consist mostly of an \(\alpha\)-helical structure (8). Interestingly, the region of annexin II that we propose contains the F-actin-binding site known to be comprised of mainly \(\alpha\)-helical secondary structure (54).

We expected that a peptide to the C-terminal 16 amino acid residues of annexin II would inhibit the binding of annexin II or AIIt to F-actin. However we found that the peptide to the C-terminal 16 amino acid residues of annexin II was unable to inhibit F-actin binding by either annexin II or AIIt (data not shown). We therefore examined the possibility that this peptide was conformationally distinct to the C-terminal region of annexin II. It was very likely that this peptide was not in the same conformation as the C-terminal region of annexin II, because circular dichroism spectra of this peptide revealed a large portion of random coil structure, with very little \(\alpha\)-helical structure (data not shown).

The inability of a peptide to the C-terminal 16 amino acids of annexin II to directly bind to F-actin presents two possibilities. First, it is possible that the C-terminal 9 amino acid residues of annexin II may only be part of the F-actin-binding site and other amino acids residues, contributed by distinct regions of annexin II, may participate in assembly of the intact site. Second, although the C-terminal region of annexin II binds F-actin, it is possible that the C terminus requires interaction with other amino acids residues to stabilize a conformation that allows these C-terminal amino acid residues to bind F-actin. Although speculative, it is reasonable to suspect that the peptide was unable to assume the expected \(\alpha\)-helical structure, because it required the presence of amino acid residues from other regions of annexin II to stabilize its \(\alpha\)-helical structure.

It was also interesting that the C-terminal truncations of annexin II most dramatically affected the F-actin binding activity of AIIt compared with the monomeric annexin II. This was an important observation, because it is AIIt and not annexin II that has been localized to the subcortical F-actin cytoskeleton. Thus, the binding of the p11 subunit to annexin II may place the C-terminal region of annexin II in a conformation that allows the 9 amino acid residues to more autonomously form the F-actin-binding site. In other words, it is possible that the proposed requirement for distal amino acids to stabilize the interaction of the 9 C-terminal amino acid residues with F-actin is minimized by the binding of p11 to annexin II.

The data obtained from x-ray crystallography is consistent with our localization of the F-actin-binding site of annexin II to the C terminus. It is known that annexin II has an overall curved, planar shape with opposing concave and convex surfaces. The convex side of the molecule is thought to face the plasma membrane and is known to contain the Ca\(^{2+}\)-binding sites and presumably the phospholipid binding domains (54). The concave face of the protein is postulated to face the cytosol and contains both the N- and C-terminal domains of the protein. It is well established that the N terminus interacts with p11 to form AIIt, and the fact that the C terminus also faces the cytosol places it in an ideal proximity to interact with the actin cytoskeleton within the cell. Thus, if AIIt is fulfilling its proposed role as a membrane-cytoskeletal linker, the C-terminal domain of annexin II appears to be in the correct orientation to bind to and potentially modulate the actin cytoskeleton.

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