Annexin B12 Is a Sensor of Membrane Curvature and Undergoes Major Curvature-dependent Structural Changes*§

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The regulation of membrane curvature plays an important role in many membrane trafficking and fusion events. Recent studies have begun to identify some of the proteins involved in controlling and sensing the curvature of cellular membranes. A mechanistic understanding of these processes is limited, however, as structural information for the membrane-bound forms of these proteins is scarce. Here, we employed a combination of biochemical and biophysical approaches to study the interaction of annexin B12 with membranes of different curvatures. We observed selective and Ca\(^{2+}\)-independent binding of annexin B12 to negatively charged vesicles that were either highly curved or that contained lipids with negative intrinsic curvature. This novel curvature-dependent membrane interaction induced major structural rearrangements in the protein and resulted in a backbone fold that was different from that of the well characterized Ca\(^{2+}\)-dependent membrane-bound form of annexin B12. Following curvature-dependent membrane interaction, the protein retained a predominantly \(\alpha\)-helical structure but EPR spectroscopy studies of nitroxide side chains placed at selected sites on annexin B12 showed that the protein underwent inside-out refolding that brought previously buried hydrophobic residues into contact with the membrane. These structural changes were reminiscent of those previously observed following Ca\(^{2+}\)-independent interaction of annexins with membranes at mildly acidic pH, yet they occurred at neutral pH in the presence of curved membranes. The present data demonstrate that annexin B12 is a sensor of membrane curvature and that membrane curvature can trigger large scale conformational changes. We speculate that membrane curvature could be a physiological signal that induces the previously reported Ca\(^{2+}\)-independent membrane interaction of annexins in vivo.

Control of membrane curvature is an important part of many cellular events (1). To better understand the molecular mechanisms by which proteins can sense or induce curvature, structural information is essential. Much has been learned from high resolution crystal structures of curvature-inducing proteins, including BAR domain proteins or epsins (2–7). In the case of BAR domain proteins, it has been shown that these proteins form highly curved helical bundles that appear to be complementary in shape to curved membranous structures. Consequently, it has been suggested that these helical bundles act as a scaffold for membrane curvature (2, 4). While this structural information is clearly important, analysis of the membrane-bound state is still necessary to fully understand the mechanisms by which proteins interact with curved membranes. For example, the N-terminal region of the BAR domain protein endophilin is known to be important for membrane interaction, yet the crystal structure does not provide any structural information for this region. In fact, this region is disordered in the absence of membranes but assumes an amphipathic \(\alpha\)-helical structure when in contact with membranes (2). Similarly, an \(\alpha\)-helical structure is also induced as curvature-sensing proteins, such as ARF-GAP and \(\alpha\)-synuclein, bind to membranes (8–11). Thus, to better understand how proteins can sense or induce membrane curvature, it is important to know how membrane curvature can affect protein structure.

In the present study we identified a novel membrane curvature-sensing property of annexin B12. Annexins are soluble proteins that reversibly associate with phospholipid membranes (12). Since annexins can be expressed, purified, and chemically labeled as well behaved soluble proteins prior to inducing membrane binding, their structures can be investigated by approaches that avoid some of the technical difficulties typically encountered in investigations of conventional membrane proteins. Crystal structures are available for soluble annexins (13–19), and the structure of the membrane-bound forms can be readily monitored by site-directed spin labeling and EPR spectroscopy (20–26). Structural studies of annexin B12 using this experimental approach therefore represent a convenient model system for studying the interplay between membrane curvature and protein structure.

Annexins are thought to play roles in several membrane-related events, such as vesicle trafficking, membrane domain organization, membrane fusion, and cell signaling (12, 27–30). Given its likely functional relevance, the membrane interaction of annexins has received considerable attention. It is now well established that annexins can interact with membranes in Ca\(^{2+}\)-dependent as well as Ca\(^{2+}\)-independent manners (20, 22, 25, 31–36). These interactions can result in very different structural and functional properties.

Reversible, Ca\(^{2+}\)-dependent membrane interaction has long been recognized as a hallmark of annexins, and the structure of...
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Annexin B12 forms a transmembrane structure (26) (Fig. 1C). The interfaceal form may be a kinetic intermediate in the formation of the transmembrane form.

To date, all structural studies of Ca<sup>2+</sup>-independent membrane interactions of annexins have been performed in vitro in model membranes at acidic pH. Nonetheless, these studies are thought to reflect physiological processes because Ca<sup>2+</sup>-independent interactions occur in intact cells. Since it is not reasonable to expect cells to regulate the interaction of annexins with membranes by global changes in cytosolic pH, it has been suggested that appropriate lipid compositions could promote such interactions at neutral pH in vivo (22). This idea is supported by in vitro experiments showing that pH-dependent membrane interaction of annexin B12 is strongly modulated by lipid composition (22). Here, we show for the first time that an annexin is a Ca<sup>2+</sup>-independent sensor of membrane curvature or curvature strain at neutral pH. Structural analysis using site-directed spin labeling as well as CD demonstrates that annexin B12 undergoes major curvature-dependent conformational changes but still retains a helical structure overall. Like the pH-dependent membrane-bound form, the curvature-dependent form also undergoes an inside-out refolding that brings buried hydrophobic residues of the solution structure into contact with the membrane. Together, these findings demonstrate that membrane curvature can have dramatic effects on protein structure and that these effects can go as far as causing a complete structural reorganization. These data raise the possibility that curved membranes or lipids with high intrinsic negative curvature induce Ca<sup>2+</sup>-independent interaction of annexin B12 with membranes at neutral pH in vivo.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—Unless otherwise noted, “annexin B12” used in this study was the recombinant protein in which the two endogenous cysteine residues were replaced with alanine (C113A/C303A) (39). The annexin B12 single-cysteine mutants K132C, F147C, and D264C and the double-cysteine mutants A77C/K265C and 113/S241C were recombinantly expressed in bacteria and purified as described previously (22, 25). The annexin B12 double mutant 255C/266C was constructed by replacing the histidine at position 255 and the threonine at position 266 in the recombinant Cys-less wild type with cysteines using site-directed mutagenesis (Strategene). The mutations were tolerated well. Co-pelleting of 255C/266C and phospholipids displayed Ca<sup>2+</sup>-dependent results that were similar to native annexin B12. Purified

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**Figure 1. Structure of annexin B12.** Annexin B12 is a monomer in solution (A) but forms a trimer upon Ca<sup>2+</sup>-dependent binding to the surface of membranes (B). The backbone folds of the soluble monomer and the membrane-bound trimer (22, 23) are similar to those observed in the crystal structure (Ref. 13; Protein Data Bank code 1AEI) of the protein. At mildly acidic pH annexin B12 refolds in the presence of membranes and forms amphipathic transmembrane helices (Refs. 20, 25, and 26; D). At intermediate pH values the amphipathic helices bind to the periphery of the membrane and form a structure (C) that may be an intermediate in transmembrane insertion (26). The red regions have a helix-loop-helix structure in the soluble monomer (A) and calcium-dependent trimer (B). However, they take up a continuous amphipathic helix in the interfacial (C) or transmembrane (D) form of the protein at low pH.
Annexin B12 mutants were stored at −70 °C in HEPES buffer (20 mM, pH 7.4) containing NaCl (100 mM) and dithiothreitol (1 mM).

**Vesicle Preparation and Gel Filtration Assay**—The following lipids were obtained from Avanti Polar Lipids (Alabaster, AL) and used for vesicle (liposome) preparation: 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-l-serine (PS) \(^3\), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC), 1,1’2,2’,5,5’-tetraoleoylcardiolipin (sodium salt) (CL), 1,2-dioleoyl-sn-glycero (diacylglycerol, DAG), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho (PA). Large unilamellar phospholipid vesicles (LUV) of glycerol, DAG, and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospholipid (PA). Large unilamellar phospholipid vesicles (LUV) of a 2:1 molar ratio for PS/PC were prepared according to the (44). Preparations of 100- and 50-nm vesicles (diameter) were prepared by repeatedly extruding (10 times) the lipid through a polycarbonate membrane of the desired size using a mini extruder (Avanti Polar Lipids). Small unilamellar vesicles (SUV) were prepared by bath sonication (three times 20 min or until the suspension was clear). Size distribution of the SUVs and extruded vesicles was then recorded by quasi-elastic light scattering (Malvern Instruments). Unless otherwise indicated, a standard binding assay was employed using annexin B12 in HEPES buffer (20 mM at pH 7.4) containing NaCl (100 mM) and dithiothreitol (1 mM) directly into a quartz cell. The background was corrected using appropriate protein-free samples.

**RESULTS**

**The Effect of Curvature on Membrane Interaction of Annexin B12**—To evaluate whether membrane curvature modulates annexin B12 membrane interaction, we developed a gel filtration assay that monitors the binding of annexin B12 to vesicles of different size and lipid composition. In this assay, all of the vesicles tested eluted at the excluded volume (~106 ml) and the soluble annexin B12 eluted at ~172 ml (data not shown). In the presence of relatively large (100-nm diameter) phospholipid vesicles (67:33 mol % PS/PC) at pH 7.4, annexin B12 eluted at fractions 24–28 (peak at ~172 ml) in the presence of EGTA (Fig. 2A, upper panel) and at fractions 14–17 (peak at ~106 ml) in the presence of Ca\(^2+\) (1 mM) (Fig. 2B, lower panel); this was determined by assaying for the presence of the protein by PAGE followed by Coomassie Blue staining. These results were expected because quantitative Ca\(^2+\)-dependent binding to phospholipid vesicles is a well established property of annexin B12 (12).

The gel filtration assay described above was used to monitor the interaction of annexin B12 with vesicles varying in size from SUV (10–36-nm diameter) to LUV (~0.5–10 μm (44)). In the presence of 1 mM Ca\(^2+\), all detectable annexin B12 co-eluted with vesicles regardless of their size (Fig. 2B), and no peak corresponding to soluble protein was observed (data not shown). A very different result was obtained in the presence of 1 mM EGTA. Here, annexin B12 bound to SUVs (Fig. 2C) but did not associate with any of the larger vesicles. Thus, annexin B12 was capable of interacting with membranes in a curvature-dependent manner that did not require Ca\(^2+\) or acidic pH.
Using the gel filtration assay, we also investigated the interaction of annexin B12 with SUVs containing various mixtures of PS and PC. As shown in Fig. 2D, the interaction of annexin B12 with these SUVs strongly depended on the PS content of the vesicles. While little or no membrane interaction could be observed with vesicles containing only PC (0% PS), increasing membrane interaction could be detected with increasing PS content (Fig. 2D). To estimate the amount of binding we quantified the amount of unbound protein using the absorbance of the unbound protein during gel filtration (data not shown). Based upon this analysis, annexin B12 binding was quantitative in the case of vesicles containing 67% PS, while vesicles containing 33%, 20%, and 0% PS yielded ~30%, 20%, and 0% binding, respectively. Dynamic light-scattering analysis showed that varying the PS content (as well as the subsequent binding of annexin B12 for a period of at least 24 h) did not produce significant changes in the diameter of SUVs (supplemental Fig. 1). Thus, as previously established for the Ca\(^{2+}\)- or pH-dependent annexin B12 membrane interactions, PS composition is important in modulating curvature-dependent membrane interaction. It should be noted that this curvature-dependent interaction is different from the previously described interaction of annexin V with mixed detergent-lipid micelles, which required Ca\(^{2+}\) (47–49).

**Structural Analysis of Curvature-dependent Membrane Interaction of Annexin B12**—To better understand the molecular mechanism that enables annexin B12 to interact with membranes in a curvature- and phospholipid composition-dependent manner, we performed a structural analysis of this membrane-bound state. All of these structural studies used SUVs with 67% PS. While the lower, more physiological concentrations of PS resulted in clearly detectable binding (Fig. 2D), the higher PS concentration was chosen to maximize the yields of membrane-bound protein and to avoid additional signals from unbound protein. First, we compared the CD spectra of annexin B12 bound to SUVs with those of annexin B12 in solution. As shown in Fig. 3, the CD spectrum for annexin B12 in solution (black trace) and bound to SUVs (red trace) are very similar, both indicating a predominantly \(\alpha\)-helical structure. These data show that curvature-dependent membrane interaction does not cause major changes in the overall secondary structure content of annexin B12. Previously, we made similar observations for the Ca\(^{2+}\)-dependent as well as for the pH-dependent membrane interaction of annexin B12 with large vesicles.

Ca\(^{2+}\)-dependent binding of annexin B12 to membranes leads to the formation of characteristic trimers on the membrane surface (Fig. 1B). To determine whether the curvature-dependent form also engages in the same type of trimer formation, we employed site-directed spin labeling and EPR spectroscopy. First, we used a derivative of annexin B12 in which the native amino acid side chain at position 132 was replaced by spin label R1 (Fig. 4, A and B) as a reporter for trimer formation. Previous studies showed that trimer forma-
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FIGURE 4. EPR analysis of annexin B12 trimer formation. A, structure of the R1 side chain. B, crystal structure of annexin B12 trimer (Protein Data Bank code 1AEI) with each monomer in a different gray tone. Position 132 (highlighted with red space filling model for each subunit of the trimer) is near the 3-fold axis of the trimer. C, EPR spectra of the 132R1 annexin B12 derivative bound to LUV in the presence of Ca\(^{2+}\) (red trace) and bound to SUV in the presence of EGTA (black). D, EPR spectrum of the 132R1 derivative at pH 7.4 in solution shown at the indicated gain. All spectra were obtained and normalized using a 200-gauss scan width. For better visualization, only the central 100-gauss portion of the spectra is shown.

FIGURE 5. EPR analysis of annexin B12 refolding using pairs of nitroxide-labeled sites. A, yellow space filling models highlight R1-labeled sites that bring two nitroxide side chains into close proximity in the in the following three doubly labeled derivatives of annexin B12: 77/265 (across the interface between domains I and IV), 113/241 (across the interface between domains II and III), and 255/266 (across helices A and B within domain IV). B, EPR spectra of the indicated annexin B12 derivatives at pH 7.4 either in solution or bound to LUV or SUV in the presence of Ca\(^{2+}\) (1 mM) or EGTA (1 mM). To generate membrane-bound annexin B12 derivatives 0.9 nmol of protein were incubated with 900 nmol of phospholipid. Prior to EPR analysis, membrane-bound protein was purified using gel filtration purified fractions as described under “Experimental Procedures.” The spectra were obtained and normalized using a 200-gauss scan width, but for better visualization, only the central 100-gauss portion of the spectra is shown. C, the inset shows the EPR spectra of 77R1/265R1 (0.2 nmol) at pH 7.4 in solution (red) or following binding to SUV (300 nmol of phospholipid) in the presence EGTA (1 mM). The EPR spectra represent the full 200-gauss scan. The graph shows the height of the central lines of EPR spectra recorded in the presence of EGTA and increasing concentrations of SUV. The lipid composition of all vesicles was PS:PC, 67:33 mol %.

that could clearly be detected in the EPR spectrum (low amplitude, broadened EPR spectral lines) of soluble annexin B12 as well as its Ca\(^{2+}\)-dependent membrane-bound form (Fig. 5B). Thus, in both of these forms, the respective residues were in close proximity, as would be expected based on the crystal structure (13). In contrast, we previously showed that the spin-spin interaction was lost upon pH-dependent membrane interaction with large vesicles, indicating that the respective sites moved apart and were separated by distances of ~20 Å or more (22). Interestingly, here we observed the analogous result for the curvature-dependent membrane interaction. In the presence of SUV and EGTA at pH 7.4, the EPR spectra of both double mutants gave rise to narrow central line widths with high intensity, a clear sign that the pronounced spin-spin interaction was lost and that the spin-labeled sites were no longer in close proximity (Fig. 5B). Thus, curvature-dependent membrane interaction, like pH-dependent membrane interaction,
must induce significant conformational changes that move the respective spin-labeled sites far apart from each other. These major conformational changes were observed even when the annexin B12 double mutants were incubated with SUVs in the presence of 1 mM Ca\(^{2+}\) (Fig. 5B), suggesting that, under these conditions, the curvature-dependent form is more stable than the Ca\(^{2+}\)-dependent form.

Experiments using the 77R1/265R1 and 113R1/241R1 annexin B12 derivatives described above showed that the domains corresponding to the four repeats of the protein underwent global structural changes in response to curvature-dependent binding to SUVs but did not address the question of whether the helices within an individual repeat rearranged. In an effort to determine whether structural reorganization occurs within an individual repeat, we prepared a new derivative, 255R1/266R1, which contains spin labels on residues located in the A and the B helices of the fourth repeat. The \(\beta\)-carbons of these two residues were separated by 7.6 Å across a helical hairpin in the crystal structure (Fig. 5A). As shown in Fig. 5B, the EPR spectra for 255R1/266R1 in solution and in the Ca\(^{2+}\)-dependent membrane-bound form were strongly broadened and of low amplitude. This result would be expected based upon the close proximity of these sites in the crystal structure and from previous studies that did not detect significant conformational changes following Ca\(^{2+}\)-dependent binding (21–23). In contrast, the EPR spectrum for the curvature-dependent membrane-bound form no longer reflected such strong spin-spin interaction, suggesting that large scale conformational changes must have occurred that rearranged the helices within the fourth repeat.

In addition to demonstrating major conformational changes, EPR spectral analysis of these double mutants also provided a convenient means by which to monitor the stoichiometry of membrane interaction of annexin B12. Using as a readout the increased amplitude of the EPR central line that occurred in 77R1/266R1 during curvature-dependent membrane interaction (inset in Fig. 5C), we studied the binding of annexin B12 as a function of increasing amounts of PS/PC containing (67:33 mol %) SUVs. As shown in Fig. 5C, increasing amounts of SUVs caused an increase in the signal amplitude until saturation occurred at \(-250\) nmol of lipid in the presence of 0.2 nmol of annexin B12. Based upon a predominant vesicle size of 16 nm and estimated areas per phospholipid as described previously (50, 51), we estimated that approximately two to three annexins bound per vesicle.

To determine whether the major conformational changes induced by highly curved vesicles resulted in lipid exposure of residues originally buried in the core of the protein, we investigated the mobility and accessibility of 147R1 and 264R1 in the crystal structure of annexin B12. Using as a readout the oxygen and NiEDDA accessibilities, \(11\text{O}_2\) and \(11\text{NiEDDA}\) determined for 147R1 and 264R1 while bound to SUV, \(\Phi\) values and depths of insertion in the vesicle bilayer were calculated from these data as described under “Experimental Procedures.”

![Annexin B12 Is a Sensor of Membrane Curvature](image)

**FIGURE 6. Oxygen and NiEDDA accessibility of 147R1 and 264R1 annexin B12.** A, the locations of positions 147 and 264 in the crystal structure of annexin B12 are highlighted with yellow space filling models. B, the EPR spectra for the 147R1 and 264R1 derivatives of annexin B12 were recorded at pH 7.4 in the presence of EGTA either in solution (red trace) or following binding to SUVs (black trace). The scan width of spectra is 100 gauss. EPR accessibility parameters \(\text{I(1)}\text{O}_2\) and \(\text{I(1)}\text{NiEDDA}\) were determined for 147R1 and 264R1 while bound to SUV. \(\Phi\) values and depths of insertion in the vesicle bilayer were calculated from these data as described under “Experimental Procedures.”
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immersion depth of nitroxide side chains (45, 46). Using this experimental approach, we estimated the immersion depth of 147R1 and 264R1 bound to SUV to be 11.2 and 13.6 Å, respectively (Fig. 6B). Thus, both of these sites exhibited considerable membrane exposure in the curvature-dependent membrane-bound form. It should be noted, however, that the immersion depth of these sites was still smaller than in the pH-dependent transmembrane form (20, 25).

Effects of Phospholipid Composition on Ca\(^{2+}\)-independent Binding of Annexin B12 to Vesicles—The packing density of phospholipid head groups within bilayers is dependent on membrane curvature and on lipid composition (1, 52, 53). Lipids with negative intrinsic curvature such as phosphatidic acid (PA), cardiolipin (CL), and diacylglycerol (DAG) can accumulate at curved regions of cellular membranes or induce curvature strain in planar bilayers (see “Discussion”). Having shown that highly curved vesicles were able to induce Ca\(^{2+}\)-independent binding and refolding of annexin B12, we sought to determine whether the inclusion of lipids with intrinsic negative curvature into vesicles of moderate diameter would induce a similar response in the protein.

We generated 50-nm diameter vesicles (see supplemental Fig. 1 for size distribution) with the following lipid compositions (mol %) and used the gel filtration assay described in the legend to Fig. 2 to monitor the interaction between vesicles and annexin B12: PS/PC (67:33), CL/PC (50:50), PA/PC (67:33), PS/PC/DAG (67:31:2). Again no interaction was detected between annexin B12 and 50 nm PS/PC vesicles but annexin B12 co-eluted with 50 nm vesicles with the other three lipid compositions. Binding was almost quantitative for vesicles containing CL/PC or PA/PC, and ~20% binding was detected in the case of DAG-containing vesicles (data not shown). However, vesicle size still played some role, as strongly reduced membrane interaction was generally observed using 1-μm diameter vesicles containing all three aforementioned lipid compositions (data not shown).

The following EPR experiments, using the 77R1/265R1 and 132R1 annexin B12 derivatives, were performed to investigate structural changes in the protein that had been induced by interaction with 50 nm vesicles containing lipids with negative intrinsic curvature. The EPR spectra of the 77R1/265R1 derivative clearly showed that 50-nm diameter vesicles containing CL, DAG, or PA induced global structural changes (Fig. 7A), and analysis of the 132R1 derivative showed no indication of trimer formation under these conditions (Fig. 7B). The EPR spectra of 77R1/265R1 and 132R1 were very similar, whether bound to highly curved PS/PC SUV (Figs. 4 and 5) or to 50-nm vesicles containing lipids with negative curvature (Fig. 7). Thus, curved membranes as well as the presence of lipids with negative curvature can induce major conformational changes in annexin B12. The ability of 2% DAG to induce such changes is particularly interesting. Using simple geometric considerations one can estimate that, on average, far less than one DAG molecule is present in an area corresponding to the size of the annexin B12 footprint (54). In addition, annexin B12 membrane interaction is substoichiometric with respect to DAG. Based on the vesicle size distribution (supplemental Fig. 1) and an average lipid area of 70 Å\(^2\) (55), we can estimate that approximatelly three to five annexin B12 molecules bind to vesicles containing on the order of ~320 – 420 DAG molecules per vesicle. As suggested previously for other membrane binding proteins (53, 56), these data are more consistent with a cumulative, curvature effect of the DAG molecules rather than a specific, high affinity interaction.

DISCUSSION

The present study shows that annexin B12 can act as a sensor of membrane curvature. The curvature-dependent membrane interaction of annexin B12 does not require Ca\(^{2+}\) or acidic pH but is strongly dependent upon vesicle size and lipid composition. The structure of the curvature-dependent membrane-bound form of the protein is radically different from that of the well characterized Ca\(^{2+}\)-dependent membrane-bound form. Following Ca\(^{2+}\)-dependent membrane binding, the backbone fold of annexin B12 is similar to that of the crystal structure of the soluble protein (22, 23), while the curvature-dependent binding induces large scale conformational changes that result in inside-out refolding. These dramatic structural changes are reminiscent of those observed previously for pH-dependent membrane interactions of annexin B12 (20, 25, 26). Together, these data demonstrate that annexin B12 has an intrinsic ability to change conformations yet retains a largely α-helical secondary structure. Membranes appear to be essential for stabilizing these alternate conformations, as no major refolding of annexin B12 has been observed in the absence of membranes, even at acidic pH (22).
Previous studies have shown that pH-dependent interaction of annexin B12 with membranes caused several helix-loop-helix motifs in the protein to refold into continuous amphipathic helices (20, 25, 26). These amphipathic helices assumed either a peripheral membrane (Fig. 1C) or transmembrane (Fig. 1D) topography, depending on the exact incubation conditions (20, 26). The curvature-induced global refolding documented herein appears to have occurred by a similar mechanism as the pH-induced refolding. Although further studies are needed to fully address this point, the data to date seem to indicate that membrane curvature stabilizes the formation of the peripherally bound form of the protein. The accessibility data for 147R1 and 264R1 clearly show that these sites are exposed to the hydrophobic region of the bilayer. The depth of membrane penetration of these sites is less than that found in the pH-dependent transmembrane form of annexin B12 and is consistent with peripheral binding.

Highly curved membranes tend to be of high energy, as curvature can interfere with optimal lipid packing interactions. Typically, lipids in the inner leaflet of curved bilayers experience a high packing density, while those in the outer leaflet experience a reduced packing density (52) (Fig. 8A). The intrinsic molecular properties of the constituent lipids can also induce curvature strain in planar bilayers (52). Some phospholipids, such as PS and PC, naturally assume cylindrical shapes with intrinsic negative curvature. Phospholipids with small head groups, such as PA, induce curvature strain in planar bilayers (52). Some phospholipids with blue head groups are cylindrical, while those with green head groups have intrinsic negative curvature inducing a curvature strain in a planar bilayer (left side). The binding of annexin B12 to the bilayer could reduce the curvature strain (right side).

The current study shows that curvature-dependent membrane interaction can cause dramatic changes in the structure of annexin B12. Since the membrane-bound form of annexin B12 can readily be investigated, this is an attractive model system with which to investigate the interplay between curvature and protein structure. In light of several reports of Ca2+-independent annexin-membrane interaction at neutral pH in vivo (62–65), it is interesting to speculate that membrane curvature, or lipids with high negative intrinsic curvature, may be involved in promoting these interactions. In this case, at least some members of the annexin family of proteins could have evolved to act as sensors of cellular membrane curvature.

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