Direct observation of Bin/amphiphysin/Rvs (BAR) domain-induced membrane curvature by means of molecular dynamics simulations

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The process of membrane curvature generation by BAR (Bin/amphiphysin/Rvs) domains is thought to involve the plasting of the negatively charged cell membrane to the positively charged concave surface of the BAR domain. Recent work [Peter, B. J., et al. (2004) Science, 303, 495–499; Masuda, M., et al. (2006) EMBO J. 25, 2889–2897; and Gallop, J. L., et al. (2006) EMBO J. 25, 2898–2910] has demonstrated the importance of the charged, crescent-shaped surface and the N-terminal amphipathic helices (present in N-BAR domains) for generating membrane curvature. These experiments suggest that curvature is generated by the synergistic action of the N-terminal helices embedding in the lipid bilayer and the charged crescent-shaped dimer acting to “scaffold” membrane curvature. Here, we present atomistic molecular dynamics simulations that directly show membrane binding to the concave face of N-BAR domains, resulting in the generation of local membrane curvature that matches the curvature presented by the BAR domain. These simulations provide direct molecular-scale evidence that BAR domains create curvature by acting as a scaffold, forcing the membrane to locally adopt the intrinsic shape of the BAR domain. We find that BAR domains bind strongly through the maximum curvature surface and, additionally, at an orientation that presents a lesser degree of curvature, thus enabling N-BAR domains to induce a range of local curvatures. Finally, we find that the N-terminal region may play a role in biasing the orientations of N-BAR domains on the membrane surface to those that favor binding to the concave face and subsequent membrane bending.

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Proteins with BAR (Bin/amphiphysin/Rvs) domains participate in various cellular processes that generate membrane curvature, such as synaptic vesicle endocytosis or the organization of the T tubule network in muscle cells (1–5). These proteins also induce curvature in liposomes in vitro, transforming them into narrow tubules (6). The recently published crystal structure for the Drosophila amphiphysin BAR domain revealed that it is a crescent-shaped dimer with a high density of positively charged residues on its concave surface (7). The shape and charge distribution of the BAR domain suggest that it induces curvature by binding negatively charged membranes to its positively charged concave surface. Although mutagenesis experiments and biophysical analysis (7–10) support this conclusion, it has not been shown that BAR domains bend membranes to locally match the curvature of their concave surface. Furthermore, because the typical curvature of BAR domain-induced tubules (with a radius of curvature of ~25 nm) does not match the intrinsic curvature of the BAR domain (radius of curvature of ~11 nm) (7), it is not clear how the local perturbation of the bilayer translates into the observed global membrane changes.

Other important questions remain regarding the role of the N-terminal helix in generating membrane curvature. Experiments have revealed that the presence of an N-terminal amphipathic helix greatly increases the ability of N-BAR domains to tubulate liposomes (7). This helix may help drive curvature by inserting into the lipid membrane in a manner similar to epsin (7, 8, 11–14). In addition, when proteins with N-BAR domains tubulate liposomes, they form visible rings on the surface of the tubule, indicating that they associate with one another on the membrane surface (6, 7, 15, 16). This association, which may be important for driving global membrane curvature changes, may also be facilitated by the N-terminal helix (12).

Results

To provide insight into these critical, experimentally unresolved questions, we have used large-scale molecular dynamics simulations to study the binding of BAR domains to lipid bilayers after initially placing the BAR domain at the membrane interface. The results in Fig. 1 correspond to two separate simulations of N-BAR domains (the simulations are hereafter designated as NBR1 and NBR2) interacting with negatively charged membranes consisting of 30% dioleoylphosphatidylserine (DOPS) and 70% dioleoylphosphatidylcholine (DOPC). The membrane extends ~45 nm in the x direction and ~10 nm in the y direction. In both simulations, the N-terminal amphipathic region is initially modeled as an α-helix embedded in the lipid bilayer. The long axis of the helix is set parallel to the surface of the bilayer and perpendicular to the long axis of the BAR domain. The helix is embedded in the bilayer leaflet at the interface between the lipid tails and lipid head groups, such that the hydrophobic residues associate with the tails, and the hydrophilic residues associate with the head groups. This arrangement corresponds to a previously proposed orientation of the amphipathic helix that would promote curvature along the same direction as the BAR domain (12, 13). Interestingly, recent experimental evidence has confirmed that the N-terminal helix does indeed embed parallel to the surface of the bilayer at the level of the phosphate groups (8).

In these simulations, we directly observed N-BAR domains inducing local curvature in the membranes by complete binding of the membrane to the concave surface of the BAR domain. Fig. 1a quantitatively compares the development of membrane curvature in the BAR domain-occupied region during the course of simulations NBR1 and NBR2 with the membrane curvature of the same lipid bilayer simulated without the N-BAR domain. Fig. 1b depicts the orientation of the BAR domains on the surface of the membrane during the course of the simulations NBR1 and NBR2. In these simulations, the N-BAR domains demonstrate two distinct modes of binding, depending on the orientation of the BAR domain with respect to the lipid bilayer. In the first case (NBR1), the concave surface of the BAR domain remains parallel to the surface of the bilayer. The membrane

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Abbreviations: BAR, Bin/amphiphysin/Rvs; DOPS, dioleoylphosphatidylserine; DOPC, dioleoylphosphatidylcholine.

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binds directly to the maximum curvature surface of the BAR domain, resulting in a very high degree of membrane curvature. In the other case (NBR2), the N-BAR domain tilts so that the maximum curvature surface of the BAR domain is no longer parallel to the surface of the bilayer. In this orientation, the BAR domain still presents a concave surface to the bilayer, but this surface has a smaller degree of curvature. This lower curvature surface, in turn, results in a lower degree of membrane curvature. Fig. 1c shows the binding of Arg and Lys residues to the negatively charged oxygen atoms on the lipid head groups during simulations NBR1 and NBR2. Interestingly, although the process of binding differs in the two cases, the final number of bound residues is quite similar. In addition, although they bind in a different orientation, the N-BAR domains in both simulations use essentially the same charged residues to bind the lipid bilayer. As expected, the initial jump in the number of bound residues coincides with, and is slightly preceded by, an abrupt increase in the curvature of the membrane. This observation is consistent with the curvature being driven by an electrostatic attraction.

Fig. 2 contains snapshots of the membrane binding and curvature development during the course of the simulations. The two systems evolve quite differently. In NBR1 (Fig. 2a), a bending mode develops directly underneath the BAR domain, whereas in NBR2 (Fig. 2c), a global bending mode forms initially. This difference in the undulation development in the two membranes may have contributed to the difference in BAR domain orientation during the simulation. After an initial development phase, both membranes bind completely to the surface that is presented to them and locally adopt the intrinsic curvature of that surface (Fig. 2b and d). Once the BAR domains are bound in these configurations, the induced membrane curvature appears to be quite stable on the time scale of these simulations.

Fig. 3 shows the average curvature and shape for the membranes and the BAR domain over the final 7 ns of the simulations, during which the membrane curvature appears to have peaked. The binding of the membrane to the maximum curvature surface of the N-BAR domain in NBR1 corresponds to the generally speculated manner of BAR domain binding; however, it results in a higher degree of local membrane curvature (peak radius of curvature of \( \approx 6.7 \) nm; Fig. 3a) than is suggested by drawing an arc along the maximum curvature binding surface.
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from 20 to 27 ns. Error bars represent the standard deviation calculated from the average membrane shape shown in b. The membrane shape is calculated by projecting the center of mass (COM) of the upper leaflet lipid head groups onto the x–z plane and averaging the COM in bins (n = 25) along the x axis. 

(c) Average curvature along the length of the BAR domain in simulation NBR1 calculated from the COM discretization (n = 8) of the BAR domain shape that is superimposed over the average atom positions in d. The number of points for the BAR domain discretization was chosen to be commensurate with the length scale of the membrane discretization. All quantities were averaged from 20 to 27 ns. Error bars represent the standard deviation calculated from 1-ns block averages.

Fig. 3. Membrane and BAR domain curvatures and geometries. (a) Average curvature along the length of the membrane for NBR1 (solid line), NBR2 (dashed line), and the plain lipid bilayer (dotted line) calculated from the average membrane shape shown in b. The membrane shape is calculated by projecting the center of mass (COM) of the upper leaflet lipid head groups onto the x–z plane and averaging the COM in bins (n = 25) along the x axis. 

(c) Average curvature along the length of the BAR domain in simulation NBR1 calculated from the COM discretization (n = 8) of the BAR domain shape that is superimposed over the average atom positions in d. The number of points for the BAR domain discretization was chosen to be commensurate with the length scale of the membrane discretization. All quantities were averaged from 20 to 27 ns. Error bars represent the standard deviation calculated from 1-ns block averages.

Fig. 4. Effect of the N-terminal region on binding orientation. Shown is the angle (θ_BAR) between the concave binding surface and the surface of the membrane for BAR domains and N-BAR domains on neutral (DOPC) or charged (DOPC/DOPS) membranes. N-terminal regions are not embedded in the membrane. The presence of the N-terminal region tends to restrict the angle of association of the BAR domain with the membrane.

Discussion

In the present work, we directly observe, by means of molecular dynamics simulations, N-BAR domains inducing curvature in membranes by binding the negatively charged moieties on the lipid head groups and causing the membrane to adopt the curvature presented by the concave surface of the N-BAR domain. These simulations reveal a larger discrepancy between the maximum local membrane curvature (radius of curvature of ~6.7 nm) and the global curvature of tubules generated by N-BAR domains (with a typical radius of curvature of ~25 nm) than was previously estimated (7). Some of this discrepancy may be due to concentration-dependent effects; liposomes incubated with much higher concentrations of N-BAR domains form vesicles that appear to have a smaller radius of curvature (7). A relatively sparse distribution of high local curvatures may translate into a smaller global curvature, which tends to approach the local curvature at higher concentrations. It would be interesting to more fully explore how the concentration of N-BAR domains on the membrane surface affects the average tubule curvature. The COPII coat proteins Sec23/24p, Sar1p, and Sec13/31p generate vesicles that match the intrinsic curvature of Sec23/24p (17, 18), although the generation of vesicles with matching curvature requires multiple interacting subunits, which makes it different from the case of N-BAR domains. However, these
observations raise interesting questions about how the cooperative generation of local curvatures by many N-BAR domains translates into global membrane shape changes.

We also observed in this work that BAR domains are able to bind membranes in various orientations that present different intrinsic curvatures to the membrane surface. For membranes that are relatively flat, the BAR domain may favor binding at an angle that presents a smaller curvature: one that more closely matches the membrane curvature. In fact, previous experimental work (7) has shown that the binding of the minimal BAR domain (without the N-terminal helix) to lipid vesicles increases as the intrinsic curvature of the vesicle approaches the intrinsic curvature of the BAR domain. In this way, the minimal BAR domain can “sense” membrane curvature. The ability of BAR domains to bind by means of a range of surfaces that present different intrinsic curvatures may increase the range of membrane curvatures that BAR domains may effectively bind (or sense). This ability could allow BAR domains to act in multiple membrane regions with different intrinsic curvatures. It could also facilitate binding in a given membrane region during multiple stages of a given curvature-generating process, with each stage defined by a different intrinsic curvature.

Finally, we observe that the N-terminal helix appears to limit the range of angles at which the N-BAR domain may interact with the membrane and tends to keep the concave surface facing the membrane. By keeping the positively charged residues facing the membrane, the N-terminal region may make it more likely that a given N-BAR domain will bind and bend the local membrane surface. This orientation may also result in more frequent membrane binding to the surface of maximum curvature, resulting in more efficient curvature induction. It is interesting to note that the width of the minimal BAR domain-binding surface is narrow: only ~30 Å. In contrast, the Sec23/24p-Sar1p complex has a much broader membrane binding region, reaching widths of 75–100 Å (17). Hence, one role for the N-terminal region may be to provide a broader base of interaction with the membrane and stabilize the “bending” orientation. It is also possible that the average orientation of N-BAR domains is a significant factor in their ability to associate with each other on the membrane surface.

**Methods**

The original coordinates for the BAR domain were obtained from Peter et al. (7) (Protein Data Bank ID code 1URU). All systems were solvated by using the TIP3P explicit water model (19) with at least 15 Å of solvent between the protein/lipid and the periodic boundary. The BAR domain structure was stable over a 60-ns simulation of the solvated BAR domain alone (~129,000 atoms; 0.1 M NaCl). The BAR domain configuration for the lipid simulations was taken from this simulation after 16.5 ns. Several solvation shells of water and NaCl were retained around the BAR domain when transferring it to the lipid systems. The original coordinates for the lipids came from the Lipid Database (20). Larger lipid systems were created from this base system of 72 lipids by first fully hydrating and equilibrating (~12 ns) the lipid patch in the constant NPT ensemble and then replicating the system along the x and y coordinate directions to create a 5 × 2 system. In addition, water, NaCl (0.1 M for DOPC systems and 0.15 M for DOPC/DOPS systems), and the BAR domain were added to the 5 × 2 lipid system, resulting in systems with initial dimensions of 251 × 98 × 149 Å (~738,000 atoms) for the nonbending studies. For mixed DOPC/DOPS systems, 30% of the phosphatidylcholine head groups from the pure DOPC system were changed to phosphatidylserine (PS) head groups to yield a nearly uniform distribution across the system. A 5 × 2 DOPC/DOPS/solvent system was run for ~40 ns and then replicated again along the x coordinate direction to generate 10 × 2 systems with initial dimensions of 477 × 97 × 156 Å (~7,380,000 atoms) for the bending studies. The CHARMM22 (21) and CHARMM27 (22) force-field parameters were used to describe the protein and lipid interactions. The parameters for the charged PS head group were generated by adding a carboxylate group to the phosphatidylethanolamine head group in CHARMM27 and by using existing CHARMM27 parameters to describe the interactions. Periodic boundary conditions were applied to all systems. The solvation, minimization, and molecular dynamics simulation protocols were performed essentially as described in ref. 23. All systems were run by using the NAMD simulation package (24) in the constant NPT ensemble [310 K and 1 atmosphere (atm) (1 atm = 101.3 kPa)] with isotropic pressure coupling for the solvated BAR domain system and fully anisotropic pressure coupling (zero surface tension) for the BAR domain plus lipid systems. Under these conditions, the area per lipid of the 10 × 2 DOPC/DOPS systems equilibrated to values between 62 and 63 Å². For further discussion of the area per lipid in these simulations, see Supporting Methods, which is published as supporting information on the PNAS website. A Langevin thermostat with a damping coefficient of 0.5 ps⁻¹ was used to maintain the system temperature at 310 K. The system pressure was maintained at 1 atm by using a Langevin-piston barostat (25) with a piston period of 2 ps and a damping time of 2 ps. Short-range nonbonded interactions were cut off smoothly between 10 and 12 Å. The particle mesh Ewald algorithm (26) was used to compute long-range electrostatic interactions at every time step. All covalent hydrogen bonds were constrained by the SHAKE algorithm (or SETTLE for water) (27, 28), permitting an integration time step of 2 fs. Analysis and system construction was primarily performed by using the CHARMM program (29), and system visualization was performed by using VMD (30).

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