Solid-State NMR Characterization of Gas Vesicle Structure

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1016/j.bpj.2010.06.041">http://dx.doi.org/10.1016/j.bpj.2010.06.041</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>Elsevier B.V.</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Wed Mar 16 00:20:29 EDT 2016</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/96087">http://hdl.handle.net/1721.1/96087</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher’s policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Solid-State NMR Characterization of Gas Vesicle Structure

Astrid C. Sivertsen,†‡ Marvin J. Bayro,†§ Marina Belenky,† Robert G. Griffin,†§ and Judith Herzfeld†*

†Department of Chemistry, Brandeis University, Waltham, Massachusetts; and ‡Francis Bitter Magnet Laboratory and §Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts

ABSTRACT Gas vesicles are gas-filled buoyancy organelles with walls that consist almost exclusively of gas vesicle protein A (GvpA). Intact, collapsed gas vesicles from the cyanobacterium *Anabaena flos-aquae* were studied by solid-state NMR spectroscopy, and most of the GvpA sequence was assigned. Chemical shift analysis indicates a coil-α-β-α-coil peptide backbone, consistent with secondary-structure-prediction algorithms, and complementary information about mobility and solvent exposure yields a picture of the overall topology of the vesicle subunit that is consistent with its role in stabilizing an air-water interface.

INTRODUCTION

Gas vesicles are buoyancy organelles that are found in a wide range of microorganisms (1). The hollow protein shells (2) are assembled and disassembled in vivo, allowing the cells to change their overall density and thereby adjust their position in the water column in response to light intensity (1) and aeration (3). Of the 14 genes in the gas vesicle operon of *Halobacterium halobium*, eight are necessary and sufficient to code gas vesicle expression, and six of these have homologs in *Anabaena flos-aquae* (4). The gas vesicle operon has most likely spread by lateral gene transfer (5), even than photosynthesis (1).

Gas vesicles are buoyancy organelles that are found in a wide range of microorganisms (1). The hollow protein shells (2) are assembled and disassembled in vivo, allowing the cells to change their overall density and thereby adjust their position in the water column in response to light intensity (1) and aeration (3). Of the 14 genes in the gas vesicle operon of *Halobacterium halobium*, eight are necessary and sufficient to code gas vesicle expression, and six of these have homologs in *Anabaena flos-aquae* (4). The gas vesicle operon has most likely spread by lateral gene transfer (5), even than photosynthesis (1). Among the other gas vesicle proteins, GvpC is the best characterized. It is about three times larger than GvpA (14) and adheres to the outside of the shell formed by GvpA, strengthening the overall structure (15). In addition to GvpA and GvpC, the proteins GvpF, GvpG, GvpJ, GvpL, and GvpM have been found in *Halobacterium salinarum* gas vesicles in amounts detectable only by immunoblotting (i.e., well under 1% of overall gas vesicle protein). GvpJ and GvpM have partial sequence similarity with GvpA and are hypothesized to be involved in cap formation, whereas GvpF, GvpL, and GvpG are hypothesized to be involved in nucleation of the gas vesicle assembly process at the tips of the caps (16). In addition, the *gypo* and *gvpk* genes are required for synthesis of gas vesicles, although GvpO and GvpK proteins have not been detected in gas vesicles, indicating that these proteins may act as chaperones in the gas vesicle assembly process.

The low-pitch helix of GvpA subunits gives rise to regularly spaced ribs running nearly perpendicular to the vesicle long axis, and it has been shown by x-ray diffraction from intact, partially aligned vesicles (11) and by atomic force microscopy (17) that the distance between neighboring ribs is 45.7 Å and that the ribs incorporate β-strands that are tilted at an angle of 36° from the vesicle long axis. This orients the H-bonds at a 54° angle relative to the vesicle axis, which is the ideal angle for mechanical stability (1), as the strength is the same in the length and width directions of the vesicle.

The hollow gas vesicles are filled with air of atmospheric composition, in equilibrium with gas molecules dissolved in the aqueous phase. Although the gas vesicle wall is permeable to H₂, N₂, O₂, Ar, CO, and CO₂, and even to a molecule as large as perfluorocyclobutane, C₄F₈ (6.3 Å in diameter), water vapor does not condense inside. This is believed to be due to a highly hydrophobic and tightly curved inner face without suitable nucleation sites (1,18). However, the details of the formation of the air-water interface are not known.

Submitted March 25, 2010, and accepted for publication June 7, 2010.
*Correspondence: herzfeld@brandeis.edu

Editor: Kathleen B. Hall.
A recent solid-state NMR study of intact, collapsed vesicles from *Anabaena flos-aquae* (19) has shown duplication of several of the most easily assigned resonances, suggesting that GvpA is a member of the growing group of metamorphic proteins (20). Using secondary-structure-prediction algorithms, the duplicated resonances were interpreted in terms of asymmetric GvpA dimers that can explain how the strands of an antiparallel β-sheet can be tilted at an angle of 36° relative to the gas vesicle axis while pairing oppositely charged side chains in salt bridges on one side of the β-sheet and leaving the other side fully hydrophobic. However, the accuracy of the predicted secondary structure remained unknown.

In this article, we describe magic-angle-spinning NMR studies that extend resonance assignments to 81% of the peptide backbone and thereby allow characterization of the secondary structure of GvpA. These extensive resonance assignments also allow site-specific characterization of mobility and solvent accessibility.

**MATERIALS AND METHODS**

**Sample preparation**

*Anabaena flos-aquae* cells were grown under a 15CO2 and 15N2 atmosphere, and the gas vesicles were isolated, stripped of GvpC, collapsed, and prepared for magic-angle-spinning experiments as described previously (19). Approximately 10 and 24 mg of sample were packed into 2.5- and 3.2-mm rotors, respectively. A 2H-exchanged sample was prepared by multiple rounds of centrifugally accelerated flotation of inflated vesicles in 99% 2H2O (Cambridge Isotope Laboratories, Andover, MA). Vesicles were then collapsed and ~28 mg of sample was packed into a 3.2-mm rotor. The solvent included 15% 2H8-glycerol (wt/wt) to prevent dehydration of the samples.

**NMR spectroscopy**

Solid-state NMR experiments were carried out using custom-designed spectrometers (D. J. Ruben, Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA) operating at 700 MHz and 750 MHz 1H Larmor frequencies, and a Bruker spectrometer (Billerica, MA), operating at 900 MHz 1H Larmor frequency. The 750 and 900 MHz spectrometers were equipped with triple-resonance 1H/13C/15N Bruker probes with 2.5-mm stators, and the 700 MHz spectrometer was equipped with a triple-resonance 1H/13C/15N Varian-Chemagnetics (Palo Alto, CA) probe with a 3.2-mm stator. The magic angle spinning (MAS) frequency was controlled to ±5 Hz using Bruker MAS controllers, and the samples were cooled with a stream of cold N2 gas during experiments, maintaining exit gas temperatures of −40°C to 5°C. Sample temperatures are estimated to be −10°C higher than the exit gas temperature, depending on the MAS frequency and the intensity of radio frequency irradiation. All spectra were referenced to external 2,2-dimethyl-2-silapentane-5-sulfonic acid (21,22).

One-dimensional spectra were acquired at 700 MHz 1H Larmor frequency, 12.5 MHz MAS, and approximate sample temperatures of +3°C and −30°C. The cross-polarization (23) (CP) and direct-polarization spectra are the averages of 64 scans each, whereas the spectra from insensitive nuclei enhanced by polarization transfer (INEPT) (24) are the average of 256 scans each. Homonuclear 13C-13C and heteronuclear 15N-13C-13C correlation spectra were acquired using various two-dimensional dipolar recoupling experiments at 5°C. A 13C-13C radio-frequency-driven dipolar recoupling (RFDR) (25–27) spectrum was recorded at 750 MHz 1H Larmor frequency and 18 kHz MAS. A 2D NCOCX spectrum was obtained at 900 MHz 1H Larmor frequency and 20 kHz MAS, using specific CP (28,29) for 15N-13C polarization transfer and 40 ms of proton-driven spin diffusion mixing (30,31) for subsequent 13C-13C transfer. A NCACX spectrum was obtained at 900 MHz 1H Larmor frequency and 20 kHz MAS, using specific CP for 15N-13C polarization transfer followed by 6.4 ms of homonuclear mixing via RFDR. Both heteronuclear experiments were recorded with 128 real and 128 imaginary points in the indirect dimension and 2048 points in the direct dimension, with dwell times of 100 μs and 12 μs, respectively, and 83 kHz two-pulse phase modulation decoupling (32) during the chemical shift evolution and detection periods. The spectra are the average of 384 transients per t1 point. A 2D INEPT-through-bond-correlation-spectroscopy (TOBSSY) spectrum was acquired at 750 MHz 1H Larmor frequency and 8.333 kHz MAS. In this experiment, 1H-13C magnetization transfer via INEPT (24) was used to excite mobile resonances, after which 5.76 ms of TOBSSY (33,34) 13C-13C mixing was applied using the P93 1H (35) with a 50 kHz 13C radio frequency field. This spectrum was acquired with 256 real and 256 imaginary points in the indirect dimension and 2048 points in the direct dimension, with dwell times of 60 μs and 12 μs, respectively, and 50 kHz two-pulse phase modulation decoupling during the chemical shift evolution and detection periods. The spectra are the average of 224 scans for each t1 point. All data sets were processed with the NMRPipe package (36) and analyzed using Sparky (37).

**RESULTS**

**Spectral quality**

Intact, collapsed gas vesicles are highly ordered molecular assemblies and present highly resolved MAS NMR spectra with average line widths of 0.5 and 0.8 ppm for 13C and 15N resonances, respectively (equivalent to full width at half-height of 80 and 70 Hz, respectively, at 700 MHz 1H Larmor frequency). These line widths are comparable to those observed for such microcrystalline proteins as BPTI (38) and ubiquitin (39), and such amyloid fibrils as those formed by alpha-synuclein (40,41), PI3-SH3 (42), and HET-s(218-289) (43), but they do not allow one-bond 13C-13C J-couplings to be resolved. Fig. 1 shows CP and direct-polarization 13C MAS spectra acquired at 3°C and −30°C. A certain degree of line broadening is observed at the lower temperature, indicating some freezing of dynamic disorder, in particular for side-chain 13C resonances.

The CP signal enhancement observed is well below the ~2.5 factor observed for rigid peptides and proteins and reveals the highly mobile nature of GvpA. This dynamic feature also manifests itself in the low intensity of many of the crosspeaks in dipolar correlation spectra, despite their narrow line widths, as can be observed in the 13C-13C spectrum of Fig. 2. However, as shown below, only a small number of residues exhibited solution-like mobility, and we therefore relied mainly on dipolar recoupling experiments for establishing intraresidue and sequential correlations.

**Sequential resonance assignments**

Narrow 15N and 13C line widths allowed us to obtain site-specific sequential resonance assignments via 2D dipolar
correlation experiments. In particular, employing CC (Fig. 2), NCACX (Fig. 3), and NCOCX (not shown) 2D correlation spectra, we were able to establish connectivities that in combination permit assignment of 15N and 13C resonances for 57 of the 70 residues, or 81% of the amino acid sequence, of GvpA. A list of assigned 15N and 13C chemical shifts is provided in Table S1 in the Supporting Material. As described and discussed earlier (19), certain resonances are duplicated, in particular those of several Gly and Ala residues, along with S49, T52, Y53, and Y56, and sequential assignment of duplicated resonances was often impeded by the relatively low intensity of these signals.

The N-terminal residue, A1, can be identified due to its unique 15N chemical shift, and sequential correlations can be established to V2 and E3. Residues K4–S8 have not been assigned because they present weak signals that severely limit interresidue correlations. The segment S9–W28 has been assigned, and it gives rise to backbone and side-chain correlations that are generally of medium intensity. The residues V29–L33 again present only weak correlation signals and are not assigned. The segment V34–V66 has been assigned sequentially, and it gives rise to backbone and side-chain signals of medium intensity for the segment V34–I47 and strong intensity for the segment A48–V66. The remaining four C-terminal residues have not been assigned, with the exception of P69, which presents weak signals that can be assigned unambiguously due to the characteristic downfield 15N chemical shift of proline. Several side-chain resonances in the main assigned segments (S9–W28 and V34–V66) either are not observed due to line-broadening dynamics or remain unresolved in highly repetitive spin systems (as expected, given 11 Ala, 8 Ile, and 10 Val residues in a 70-residue protein).

Although backbone 13C and 15N resonances tend to present significant degeneracy, it was possible to resolve assignment ambiguities in many instances by relying on

FIGURE 1 13C MAS spectra of gas vesicles acquired at 700 MHz 1H Larmor frequency and 12.5 kHz MAS frequency, using cross-polarization (A and B) and direct polarization (C and D) at 3°C (A and C) and −30°C (B and D). The relatively low cross-polarization enhancement over direct excitation indicates considerable mobility in the protein. Each spectrum is an average of 64 scans recorded with recycle delays of 3 s and 30 s in CP and DP experiments, respectively. A glycerol resonance is visible at ~75 ppm in D.

FIGURE 2 13C-13C correlation spectrum acquired at 750 MHz 1H Larmor frequency, 18 kHz MAS frequency, and 5°C. The experiment used 3.52 ms of RFDR mixing to establish homonuclear connectivities. Cross-peaks corresponding to CA-CB correlations for several assigned residues are indicated.

FIGURE 3 13C region of an NCACX spectrum acquired at 900 MHz 1H Larmor frequency, 20.0 kHz MAS frequency, and 5°C. The experiment used 15N-13C cross-polarization followed by 13C-13C RFDR mixing to establish heteronuclear connectivities. Labels correspond to N-CA correlations, unless otherwise noted.
side-chain correlations in NCACX and NCOCX spectra. Resonance intensities vary widely across the GvpA sequence, such that certain parts of the amino acid sequence, especially the C-terminal third, are predominant in the $^{13}$C-$^{13}$C and $^{15}$N-$^{13}$C correlation spectra. The segments S9–W28 and V34–I47 generally have weak- or medium-intensity correlations, but especially weak resonances arise from residues such as I15, L19, I36, E37, L38, L39, E42, and R44. The segment A48–V66 generally gives rise to correlations with high intensities, except for the duplicated residues Y53 and Y56, the duplicate form of T52, and residues V60, L62, and T63, which give rise to resonances of moderate intensity.

The side chains beyond CB of several residues in the GvpA sequence are observed in full (in the combined spectra, not necessarily in just one type of spectrum). These residues are L11, V14, I15, I18, I23, V24, I25, L38, L39, V46, I47, V50, T52, V60, L62, and T63. On the other hand, incomplete side chains are observed for I36, V34, I41, I45, and L54. For almost all the acidic residues (Glu and Asp), carboxyl correlations are missing in most spectra. Among the three of each in the sequence, only one Arg and one Lys side chain are observed in full. The Y53 side chain readily shows up in full in RFDR and spin-diffusion spectra with short mixing times, whereas longer mixing times are required for the entire Y56 side chain to be observed. This dissimilarity is likely the result of different ring dynamics in the two Tyr residues. The ring system of W28 has not been observed in full; only a CB-CG correlation has been observed in $^{13}$C-$^{13}$C spectra with long mixing times.

After assignment to the degree possible, two Ser, one Val, one Glu, and eight Ala CA-CB correlations in RFDR spectra remain unassigned. The Ser, Val, and Glu correlations could come from unassigned parts of the protein, but only one Ala remains unassigned in the sequence. Therefore, the rest of the Ala CA-CB resonances are likely to be peak duplications. In the CA regions of NCACX and NCOCX spectra, another ~20 residues could be assigned to the yet unassigned peaks. This number is again higher than the actual number of unassigned residues, indicating duplications that are more extensive than the ones assigned so far.

**Secondary-structure analysis**

Values of the Ca-Cβ (44) secondary chemical shifts (45,46) are shown in Fig. 4 A, and they display strong agreement with the PSIPRED secondary-structure prediction shown in Fig. 4 B. A TALOS (47) chemical shift analysis of the assigned residues gives the secondary-structure pattern shown in Fig. 4 C, which largely agrees with the secondary chemical shift result and with the PSIPRED prediction. TALOS results are only shown for one form for the residues that display peak duplications, as the TALOS results for the two forms of GvpA do not differ by more than 3–4°, which is within the standard deviation of the TALOS output.

The N-terminal α-helix is predicted by PSIPRED to extend from L11 to I18, and both secondary chemical shifts and the TALOS calculation indicate the beginning of the helix at L11. However, our chemical shift analysis indicates that the helix may continue until D20. It is worth noting that the T5–R17 fragment that has been observed in digestion studies (13) corresponds to cleavage at this transition between the N-terminal α-helix and the N-terminal β-sheet that follows. The latter is predicted by PSIPRED to extend from V24 to V34, and the secondary chemical shifts and TALOS results indicate that it begins at I23 and continues for a few residues. Resonance assignments were not obtained for the second half of this sequence, but there is a secondary chemical shift for V34 that has a β-sheet value. According to our chemical shift analysis the C-terminal β-sheet extends from I36 to S49, whereas the segment V50–A59 adopts an α-helical conformation, in close agreement with PSIPRED. It is predicted that the remainder of the sequence adopts a coil conformation, and neither secondary chemical shifts nor TALOS indicate any significant stretches of defined secondary structure for this part of the protein. TALOS results show nonstandard angles for five residues: K21, G22, G35, V60, and G61. Because of their large conformational flexibility, it is not surprising that atypical φ and ψ values are observed for Gly residues, especially as they are found in intermediate regions. The unusual torsion angles of Gly neighbors K21 and V60 are also consistent with the Gly residues found in turns between the secondary-structure elements. The Ramachandran plot in Fig. 5 shows that the assigned residues reside in the allowed α-helix and β-sheet regions, except for the few mentioned above.

**Amphipathic α-helices**

Fig. 6 shows nearest-neighbor-corrected (46,48) secondary $^{15}$N backbone chemical shift values for the α-helical segments of GvpA. As all residues in the figure are α-helical, the differences in $^{15}$N chemical shifts are mainly governed by differences in NH-CO hydrogen-bond length along the helix, where large secondary chemical shifts indicate short NH-CO hydrogen bonds (45,49). The plots display variations of the secondary chemical shifts, with a period of roughly three to four residues, corresponding to an α-helical turn. A similar periodicity is found in amide proton resonances of curved amphipathic helices, where backbone hydrogen bonds are shorter on the hydrophobic side and longer on the hydrophilic side (46,50). Since amide $^{13}$N chemical shifts exhibit trends with respect to hydrogen-bond length that are similar to those of amide $^1$H shifts, we may speculate that the periodicity observed in the $^{15}$N shifts for the N-terminal helix (Fig. 6, left side) might be the result of curvature in the helix, whereas the trend in the C-terminal helix is less defined. These observations are consistent with the
amphipathic residue patterns seen in helical wheel renderings (see Fig. S1).

Molecular mobility

Fig. 7 shows $^1$H-$^{13}$C INEPT spectra acquired at $3^\circ$C (Fig. 7A) and $-30^\circ$C (Fig. 7B). In the solid state, the INEPT sequence excites those $^{13}$C sites with sufficient flexibility to average out the dipolar interactions to the point that they do not interfere with the much weaker J-couplings. Typically, only mobile side chains and highly flexible backbone regions give rise to INEPT signals. As Fig. 7 shows, a few signals are observed at $3^\circ$C, in most cases corresponding to single sites. About eight CA sites are observed, indicating that at this temperature most of the backbone is not very flexible. The number of side chains corresponds roughly to the number of backbone sites. At $-30^\circ$C, there is much less mobility, and no CA sites are observed, whereas some side chain sites are still observed, mostly methyl groups. The spectra in Figs. 1 and 7 together indicate that GvpA in intact collapsed vesicles is overall moderately dynamic, but presents only a few sites with solution-like mobility.

To assign the highly mobile residues, a 2D $^{13}$C-$^{13}$C INEPT-TOBSY spectrum was acquired (Fig. 8). In this experiment, after excitation of mobile $^{13}$C sites, TOBSY mixing is applied to achieve homonuclear polarization transfer mediated by $^{13}$C-$^{13}$C J-couplings. From the nearly complete $^{13}$C spin systems in these data, we were able to identify three Ala, two Val, and one Pro set of resonances that are also observed, at least weakly, in dipolar correlation spectra. In addition, resonance sets were detected for Ser, Thr, Leu, Ile, Glu, Gln, Lys, and Arg. Except for the unique proline, P69, none of the observed INEPT-TOBSY resonances could be assigned sequentially. However, the Thr resonances are likely to correspond to T5, since T52 and T63 are already assigned in dipolar correlation spectra.

Solvent accessibility

To gain information about the GvpA subunit topology that forms the gas vesicle wall, we acquired water-edited correlation spectra and studied a $^2$H$_2$O-exchanged sample.
Water-edited spectra can be used to identify solvent-accessible residues by delaying $^1$H magnetization transfer to $^{13}$C until the intrinsic $^1$H polarization in the protein is relaxed by relatively strong dipolar interactions. However, the spectra we recorded in this fashion show that considerable $^1$H polarization remains in the protein after the filter period, possibly due to motion of certain segments of the polypeptide chain. Therefore, water-edited 1D and 2D $^{13}$C-$^{13}$C correlation experiments yielded ambiguous information.

On the other hand, a sample in $^2$H$_2$O allowed the identification of exchangeable amide sites by analyzing $^{15}$N-$^{13}$C double-CP spectra recorded with different contact times for the initial $^1$H-$^{15}$N CP step. Short $^1$H-$^{15}$N CP periods are sufficient to transfer magnetization only to $^{15}$N sites with directly bonded protons, whereas longer CP times allow polarization to build up at neighboring sites. Fig. 9 shows NCA spectra of vesicles collapsed in $^2$H$_2$O recorded with $^1$H-$^{15}$N CP contact times of 0.5 ms (Fig. 9 A) and 8.0 ms (Fig. 9 B). Most NCA crospeaks appear at both CP times, but certain peaks that are not observed at 0.5 ms CP time are observed with an 8-ms contact time, including: A1, V2, T52, V60, T63, and several unassigned NCA peaks, some of which appear to correspond to residues with doubled resonances. In a similar way, although Lys Nz-Ce correlations are observed at 8 ms CP time, they are not seen at 0.5 ms CP time (not shown), and thus we can conclude that all three Lys side chains are solvent-accessible. On the other hand, since the majority of NCA correlations are observed at 0.5 ms (Fig. 9 B), most amide sites do not exchange with $^2$H$_2$O, either due to participation in secondary-structure hydrogen bonding, or because they are buried within the solvent-inaccessible protein core.
DISCUSSION

GvpA subunit structure

Solid-state NMR assignments for 81% of the GvpA sequence support the coil-α-β-α-coil secondary structure that is predicted for this protein. This new information makes it necessary to reject the so-called paperclip model of gas vesicle subunit structure (1), which assumed GvpA to be in an all-β-sheet conformation. As seen in Fig. 4, the β-sheet region has a maximum length of only 27 residues, and is flanked by α-helices at both ends. It is also clear that the termini beyond the helices are in coil conformation and solvent-accessible, as indicated by $^2$H$_2$O exchange at certain sites near the termini.

Air-water interface formation

Gas vesicles function by forming an interface between the aqueous phase and the air inside the vesicle. In contrast, ordinary proteins function fully immersed in condensed aqueous or lipid phases, and tend to unfold on exposure to an air-liquid interface (51,52), with the hydrophobic core exposed to the air. On the other hand, extended β-sheets with an alternating polar-nonpolar amino acid sequence are stable at an air-water interface, as their amphipathic nature allows them to expose the hydrophobic side chains on one face to the air, and the hydrophilic side chains on the other face to the water. Indeed, amyloid fibrils have been found to self-assemble in air-water interfaces, with their hydrophobic side chains exposed to the air (53). Interfacial amyloids have even been found to occur naturally: the soil bacteria Streptomyces coelicolor secrete chaplin proteins (54) and filamentous fungi secrete hydrophobins (55), both proteins that self-assemble into amyloid-like fibrils at the air-water interface, thereby lowering the surface tension of water and presumably facilitating the growth of spore-forming hyphae into the air.

Gas vesicles have two segments that our NMR data indicate to be of β-sheet secondary structure, as predicted. Both segments have alternating polar-nonpolar residue patterns that are highly conserved, although they are otherwise known to be disfavored by nature because of their propensity for amyloid formation (56). It is likely that this portion of GvpA is largely responsible for the air-water interface, as the presence of an inward-facing hydrophobic surface would explain not only the ability of gas vesicles to prevent internal condensation of water, but also the strong 10 Å reflection, typical of stacked β-sheets, that is observed in x-ray diffraction studies of collapsed, but not of inflated, gas vesicles (11).

Perhaps the most intriguing aspect of the functional amyloids is how cells prevent these molecules from accumulating in dysfunctional deposits. In particular, how do aquatic organisms control the assembly-disassembly of gas vesicles and the recycling of GvpA subunits? Chaperones (possibly the GvpO and GvpK proteins that are essential for gas vesicle expression, but are not detected in gas vesicles) are likely to be involved in the process, and the presence of the amphipathic α-helices in GvpA may help prevent catastrophic aggregation and precipitation. A similar issue arises in the secretion of chaplins and hydrophobins.

CONCLUSIONS

We have obtained well-resolved solid-state NMR spectra of intact, collapsed gas vesicles from Anabaena flos-aquae, allowing assignment of 81% of the GvpA sequence, followed by secondary chemical shift analysis, indicating a coil-α-β-α-coil secondary structure. Nearest-neighbor corrected $^{15}$N secondary shifts provide evidence for curvature in the α-helices and $^2$H$_2$O exchange experiments identify solvent-exposed residues. These data provide insights into the topology of GvpA subunits in the gas vesicle shell and how it might stabilize the air-water interface. However, distance constraints are needed to obtain a structural model.

SUPPORTING MATERIAL

A table of resonance assignments for GvpA (Table S1) and helical wheels for the α-helices of GvpA (Fig. S1) are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00784-8.

The authors thank Drs. Patrick van der Wel and Anthony Bielecki for helpful discussions and technical support.

Funding for this work was provided by National Institutes of Health grants EB002175, EB001960, and EB002026.

REFERENCES
