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Investigating the interaction between peptides of the amphipathic helix of Hcf106 and the phospholipid bilayer by solid-state NMR spectroscopy

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Abstract

The chloroplast twin arginine translocation (cpTat) system transports highly folded precursor proteins into the thylakoid lumen using the protonmotive force as its only energy source. Hcf106, as one of the core components of the cpTat system, is part of the precursor receptor complex and functions in the initial precursor-binding step. Hcf106 is predicted to contain a single amino terminal transmembrane domain followed by a Pro-Gly hinge, a predicted amphipathic α -helix (APH), and a loosely structured carboxy terminus. Hcf106 has been shown biochemically to insert spontaneously into thylakoid membranes. To better understand the membrane active capabilities of Hcf106, we used solid-state NMR spectroscopy to investigate those properties of the APH. In this study, synthesized peptides of the predicted Hcf106 APH (amino acids 28–65) were incorporated at increasing mol% into 1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine (POPC) and POPC/MGDG (monogalactosyldiacylglycerol; mole ratio 85:15) multilamellar vesicles (MLVs) to probe the peptide-lipid interaction. Solid-state ³¹P NMR and ²H NMR spectroscopic experiments revealed that the peptide perturbs the headgroup and the acyl chain regions of phospholipids as indicated by changes in spectral lineshape, chemical shift anisotropy (CSA) line width, and ²H order S_{CD} parameters. In addition, the comparison between POPC MLVs and POPC/MGDG MLVs indicated that the lipid bilayer composition affected peptide perturbation of the lipids, and such perturbation appeared to be more intense in a system more closely mimicking a thylakoid membrane.

Keywords

amphipathic helix; membrane active peptide; twin arginine transport; chloroplast TatB

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1. Introduction

In plant cells most thylakoid lumen proteins are synthesized in the cytosol as higher molecular weight precursors containing targeting sequences, which allow import into chloroplasts and localization to thylakoid lumen for function [1, 2]. Two thylakoid transport systems, homologous to bacteria export systems, direct precursors to the thylakoid lumen [3]. The chloroplast Sec system transports largely *unfolded* proteins across the membrane in an ATP dependent manner, while the chloroplast twin arginine translocation (cpTat)² system delivers highly *folded* proteins across the membrane utilizing the trans-thylakoidal protonmotive force as its only energy source. Another distinguishing feature of Tat transport system is the presence of a twin arginine amino acid motif in the lumen-targeting signal peptide of the precursor, which gives the pathway its name. The cpTat pathway consists of three core membrane-bound subunits, Tha4, Hcf106, and cpTatC; whereas the homologous proteins in the bacterial Tat system are TatA, TatB and TatC [2]. Sequence analysis predicts that Tha4 (TatA) and Hcf106 (TatB) have similar structure, both consisting of an N-terminal transmembrane domain (TMD), followed by an amphipathic helix (APH) and an unstructured C-terminus (C-tail) [4], however, the APH domain and C-tail region of Hcf106 are approximately two times larger than those of Tha4. The predicted structure for Tha4 has been confirmed by determination of the structure of a truncated version of the homologous protein, TatA_d (2–45), from *Bacillus subtilis* by solution NMR [5] and solid-state NMR [6]. Even though Tha4 and Hcf106 appear to have structural similarities, this has not been investigated directly. In addition the two proteins are functionally distinct such that one cannot functionally replace the other [4]. cpTatC is an integral membrane protein with six transmembrane-spanning helices [7, 8]. Nuclear genes encode all three proteins, and they are synthesized in the cytoplasm as larger molecular weight precursors containing N-terminal amino acid extensions, the stromal-targeting transit peptides. cpTatC is localized to the thylakoid via a stromal intermediate [9], and it cannot integrate into the thylakoid membrane directly. Conversely, Tha4 and Hcf106 do not appear to use a stromal intermediate, and they can integrate directly into thylakoid membranes even in the absence of their signal peptide [4]. Currently how these proteins are able to insert into the membrane spontaneously or unassisted is unknown. Here we investigated interactions of a synthetic peptide corresponding to the APH region of Hcf106 for its ability to interact with multi-lamellar vesicles (MLVs) as insight into the ability to spontaneously insert into thylakoid membranes. Understanding Hcf106 interaction with lipid bilayers may also provide insights into Hcf106 function and activity.

One way to better understand the integration, topology, and structure-function relationships of membrane proteins is to study the interaction between the peptide and lipids by solid-state NMR spectroscopy. Solid-state NMR techniques are widely used to study the structural and dynamic properties of peptides and lipids [10–13]. ³¹P and ²H solid-state NMR spectroscopy are useful probes to study peptide-lipid interactions from the perspective of lipids. Utilizing ³¹P as a natural probe, which is sensitive to the conformation of lipid headgroup, lipid phase and electrostatic property, ³¹P NMR is extensively used to investigate the dynamics and interaction between lipid headgroups with peptides [14–16]. Complementary to ³¹P NMR, ²H solid-state NMR spectroscopy provides information on the orientation and dynamics of the acyl chains of lipids by incorporating peptides or proteins into acyl-chains deuterated phospholipid bilayers [17–19]. Peptide-lipid interactions are based on the amino acid sequence of proteins and the composition of lipids, and are widely used to address questions such as protein and lipid structure, hydrophobicity and aggregation

²Abbreviations used: cpTat, chloroplast twin arginine transport; TMD, transmembrane domain; APH, amphipathic α -helix; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine; MGDG, monogalactosyl diacylglycerol; MLV, multilamellar vesicles; CSA, chemical shift anisotropy; SCD, segmental C-D bond order parameter.

of proteins, charge distribution on lipid, depth of peptide in the lipid milieu, modulation of interaction by altering the lipid composition [20–23]. Unlike small micelles with high curvature, which could hardly retain native structure of peptides, multilamellar vesicles (MLVs) are more membrane-mimicking systems that are composed of multi-layers of lipid bilayers [24, 25].

Here we use solid-state NMR spectroscopy to investigate the topology of vesicles from the perspective of the lipids, obtaining preliminary topology information of Hcf106 in lipids by studying the peptide-lipid interaction. To simplify the study of this 176 amino acid protein, a peptide encompassing the APH (residues 28–65) of Hcf106 were synthesized by Fmoc-based solid-phase methods, and purified by high-performance liquid chromatography [26]. These peptides were then incorporated into POPC (1-palmitoyl-2-oleoyl-sn-glycero-phosphoatidylcholine) and POPC/MGDG (monogalactosyldiacylglycerol) vesicles at various concentrations. The synthetic phospholipid bilayers mimicked biological membranes providing a controlled environment in which to assay peptide interaction or insertion with the membrane. A peptide from the C terminus of the small subunit of Rubisco (mSSU Rubisco) was predicted to be non-amphipathic by standard algorithms, and was used as a control peptide in this study. Using ^{31}P NMR spectroscopy we showed that Hcf106 APH peptides interact with phospholipid headgroups. In addition ^2H NMR spectroscopic measurements revealed the perturbation of the deuterated acyl chains by the presence of peptide and the effect of lipid composition on the peptide-lipid interaction. Further, the order parameter S_{CD} from ^2H NMR demonstrated the dynamic characteristics of lipid headgroup and acyl chain and the effect of peptide addition to the vesicles. These experimental results confirmed the membrane-associate activity of the proposed amphipathic helix of Hcf106 and suggested that Hcf106 mostly sat on the membrane surface and perturbed the hydrophobic region of phospholipid to a lesser extent.

2. Materials and Methods

2.1 Materials

Synthetic phospholipids POPC and POPC- d_{31} were purchased from Avanti Polar Lipids (Alabaster, AL). Natural galactolipid from parsley leaves, MGDG, was purchased from Larodan Fine Chemicals AB (Sweden). All lipids were dissolved in chloroform or chloroform/methanol and stored at -20°C under N_2 gas until use. Fmoc amino acids were purchased from Novabiochem (San Diego, CA). Peptide synthesis resin and other chemicals for synthesis were purchased from Applied Biosystems (Foster City, CA). HPLC grade acetonitrile was purchased from VWR (Radnor, PA) and was filtered through a 0.22-mm nylon membrane before use. Water was purified using a Nanopure reverse osmosis system (Millipore, Bedford, MA). Chloroform, piperidine, EDTA, N-[2-hydroxyethyl] piperazine-N''-2-ethane sulfonic acid (HEPES) and 2,2,2 trifluoroethanol (TFE) were purchased from Sigma-Aldrich (Milwaukee, WI). The deuterium-depleted water was purchased from IsotecTM (Miamisburg, OH).

2.2 Synthesis and purification of peptide Hcf106 APH and peptide mSSU Rubisco

Hcf106 APH and mSSU Rubisco were synthesized using Fmoc solid-phase peptide synthesis chemistry on a CEM microwave peptide synthesizer (Matthews, NC). The sequence for the amphipathic helix of Hcf106 is NH_2 -LAEVARNLGKTLREFQPTIREIQDVSREFKSTLEREIG-COO⁻, while the sequence for the control peptide mSSU Rubisco is NH_2 -EHNKSPRYDGRYWTMWKLPFGTTDASQVLG-COO⁻. After the peptides were cleaved from their solid support, they were purified via HPLC on a C18 reverse-phase preparation column. The column was equilibrated with 95% solvent A (100% H_2O) and 5%

solvent B (60% acetonitrile, 40% H₂O for Hcf106 APH; 90% acetonitrile, 10% H₂O for mSSU Rubisco), and was eluted with a linear gradient of 5% to 95% solvent B. The buffers also contained 0.1% trifluoroacetic acid (TFA). The purified peptide was lyophilized to remove existing solvent. Purity was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry to ensure the purity of the peptides.

2.3 NMR sample preparation

POPC bilayers containing Hcf106 APH peptide of various concentrations were prepared as described [15]. Briefly Hcf106 APH peptide (2.0mg–6.1mg) and POPC bilayers (17.5mg) were dissolved in 2,2,2 trifluoroethanol and chloroform, respectively, and the peptide was added to the lipids at the mol% indicated in the Figure Legends. The mixtures were dried under steady N₂ gas and placed in a vacuum desiccator overnight to remove residual solvent. The multi-lamellar vesicles (MLVs) were prepared by suspension of the dried mixture in 95μl HEPES buffer (30mM HEPES, pH 7.0, 5mM EDTA, 20mM NaCl) followed by five cycles of moderate vortexing for two min, freezing with liquid N₂ for one min and sonication for five min in a 45°C bath sonicator. While the presence of residual TFA from the cleavage reaction should be minimized, the presence of residual TFA would lower the pH of the buffer, especially given the small volume of dilute buffer added; therefore, the peptide solution may have a pH <7.0. The fully homogenized samples were transferred to NMR sample tubes. Peptide incorporation into POPC/MGDG MLVs (85%:15% ratio) were prepared following the same procedure, maintaining the total lipid amount at 17.5 mg. For the ²H NMR experiments, 5mg POPC-d₃₁ phospholipids replaced the same amount of non-deuterated POPC in the sample to maintain total lipid amount (17.5mg). POPC and POPC/ MGDG MLVs containing 6 mol % control peptide from the SSU of Rubisco (5.3mg) were prepared by the same procedure.

2.4 NMR spectroscopy

All NMR data were recorded on a Bruker Avance 500MHz WB NMR spectrometer equipped with a CPMAS probe (Bruker, Billerica, MA). ³¹P NMR spectra were acquired at 202.4MHz with proton decoupling using a 4 μs 90° pulse and a 4 s recycle delay. For the ³¹P static NMR spectra 512 scans were taken and the free induction delay was processed using 200 Hz of line broadening. The spectral width was set to 500 ppm. All ³¹P NMR spectra were referenced by assigning the 85% H₃PO₄ ³¹P peak to 0 ppm.

²H NMR spectra were performed at 76.77 MHz employing a quadrupolar echo pulse sequence using quadrature detection with complete phase cycling of the pulse pairs. The 90° pulse length was 4 μs, the interpulse delay was 40 μs, the recycle delay was 0.4 s, and the spectral width was set to 100 kHz. For the ²H static NMR spectra 122,880 scans were taken and were processed using 200 Hz of line broadening. Powder-type ²H NMR spectra were deconvoluted (dePaked) using the algorithm of McCabe and Wassall [27] such that the bilayer normal was perpendicular with respect to the direction of the static magnetic field. The quadrupolar splittings were measured from the dePaked spectra, and the ²H order parameters S_{CD}ⁱ were calculated according to the equation $S_{CD}^i = \Delta\nu_i / (3/2(e^2qQ/h))$, where S_{CD}ⁱ is the quadrupolar splitting for a deuteron attached to the *i*th carbon of the POPC acyl chain, and e²qQ/h is the quadruple coupling constant (168 kHz for deuteron in C-D bonds). The order parameters of the methyl groups at the terminal acyl chain were multiplied by three as the calculated values [28].

3. Results and Discussion

3.1 ^{31}P NMR studies indicate Hcf106 APH interaction with MLVs

Chloroplast Tat component Hcf106 is predicted to contain a N-terminal proximal amphipathic α -helix (Figure 1A), which has not been characterized experimentally. In addition, previous studies suggest that Hcf106 has the ability to insert into the thylakoid via the spontaneous pathway, requiring no membrane bound protein assistance or additional energy, either in the form of nucleotide triphosphate hydrolysis or transmembrane protonmotive force [4]. To better understand the ability of Hcf106 to interact with membranes, we investigated the membrane interactivity of one of the key predicted membrane active components, the APH, using a synthetic peptide (Figure 1A). Helical wheel projections of the APH peptide used in this study clearly indicate the presence of a hydrophilic face and a hydrophobic face of the helix (Figure 1B).

To investigate the nature of lamellar and nonlamellar phase transitions exhibited by the POPC MLVs in the presence of the Hcf106 APH peptide, static ^{31}P NMR spectroscopic measurements were made (Figure 2A). The Hcf106 APH peptide concentration was varied from 0 mol % to 6 mol % with respect to POPC lipids. As expected for POPC bilayers at a temperature above the chain melting transition temperature (T_m) of -2°C [29], all spectra displayed a motionally averaged powder-pattern at 25°C , indicating that the phospholipid bilayers are in the liquid crystalline phase (Figure 2A). Increasing amounts of Hcf106 APH peptide incorporated into POPC MLVs caused a corresponding increase in the static lineshape width of the ^{31}P NMR spectra as compared to the lineshape width of the empty POPC vesicles (Figure 2A). Furthermore, the addition of the peptide caused a corresponding decrease in the chemical shift anisotropy (CSA, $\sigma_{\parallel} - \sigma_{\perp}$) line widths. CSA line widths were measured for control vesicles and vesicles containing 6 mol% APH peptide (Figure 2A) and were found to be 45 ± 1 ppm and 42 ± 1 ppm, respectively. There was a 3 ppm decrease in CSA line width when 6 mol % peptide was added as compared to control POPC bilayers, indicating that the headgroup region of the phospholipid bilayer was perturbed by the addition of Hcf106 APH peptide. This change contrasts directly with a CSA line width measure of 45 ± 1 ppm in the presence of 6 mol% control peptide (Supplemental Figure 1A), which was indistinguishable from control vesicles.

The Hcf106 APH peptide is derived from the thylakoid localized Hcf106 protein. Thylakoid membrane has a very high proportion of nonphospholipids to phospholipids as well as a high proportion of the nonbilayer forming lipid, monogalactosyl diacylglycerol (MGDG) that can compose $>50\%$ of thylakoid lipid [30]. To more closely mimic the native thylakoid lipid composition, we tested Hcf106 APH interaction with vesicles containing 15 mol% MGDG. In this synthetic system, we were able to easily generate vesicles containing 15 mol % MGDG (85:15 mole ratio POPC:MGDG), but higher concentrations yielded unstable vesicles and were not used for this study. When increasing amounts of the Hcf106 APH peptide were incorporated into POPC/MGDG MLVs, the corresponding ^{31}P lineshape broadened compared to that of control POPC/MGDG MLVs (Figure 2B). As with POPC MLVs, the CSA widths decreased as more peptide was added to the POPC/MGDG MLVs. The CSA widths for control POPC/MGDG MLVs and MLVs containing 6 mol % APH peptide were 45 ppm and 41 ppm, respectively. There was a 4 ppm decrease in CSA width when 6 mol % peptide was added when compared to the empty vesicles. However the presence of the control peptide with POPC/MGDG vesicles resulted in a CSA line width of 45 ± 1 ppm, the same as control vesicles (Supplemental Figure 1B). The most obvious difference in ^{31}P spectra lineshape between the POPC MLVs and the POPC/MGDG MLVs was the appearance of an isotropic peak that increased in intensity as the concentration of Hcf106 APH was increased (Figure 2B). The presence of such an isotropic peak suggests

that smaller vesicles were induced to form due to the presence of the APH peptides and the contribution from the isotropic phase increased as more Hcf106 APH was incorporated [31].

At least three possible occurrences can explain this change in CSA line width, (1) increased stochastic fluctuations experienced by the lipid head groups due to an increase in the disorder of individual lipids caused by the increasing presence of the APH, but not the control, peptide; (2) increased axial rotational motion as well as lateral diffusion rate of lipid upon peptide incorporation; or (3) increased dynamics of the vesicles, resulting from the interaction between the peptide and head groups of the phospholipids [13, 32–34]. However, an increase in the long axis rotation of the lipid would be unlikely to affect the CSA line width because at the point at which the ^{31}P CSA line width becomes axially symmetric it has reached an average and increasing the rotation at that point will not change those averages. In addition, an increase in the lateral diffusion rate of the lipid is also unlikely to be large enough to affect the CSA line width. More likely the change in CSA line width is due to an increase in the disorder of the individual lipids as the amount of the APH, but not the control, peptide increases. For example, a decrease in the CSA line width may correspond to a change in the orientation of the head groups of the lipid such that they are less upright (i.e., the angle ϑ_x of the chemical shift tensor increases from the lipid axis of rotation perpendicular to the plane of the phosphorous and oxygens [35]). Regardless, the shift in CSA when the APH peptide was present indicates a perturbation of the liquid crystalline array of the lipids, and a change in averaged orientation of the lipid head groups with respect to the bilayer normal, induced by rotational motion of lipid head group [32, 36]. Regardless, the APH peptide interacted with MLVs containing the thylakoid-specific lipid, MGDG more intensely as compared to MLVs containing only POPC.

3.2 ^2H NMR studies indicate perturbation of POPC and POPC/MGDG lipids acyl chain upon Hcf106 APH incorporation

Because static ^{31}P NMR spectra only reflects the perturbation of the peptide to the phospholipid headgroup, ^2H solid-state NMR spectroscopy was utilized to investigate the effect of increasing concentration of Hcf106 APH peptide on the order and dynamics of the acyl chain of POPC- d_{31} phospholipid in POPC and POPC/MGDG MLVs (Figure 3). All spectra display overlapping doublet resonances with the largest splitting resulting from the C-D bond closest to the glycerol backbone and the central splitting arising from the terminal methyl group [13, 31, 34]. Clearly, addition of APH peptide at various concentrations altered the line shape and resolution of ^2H NMR spectra of the POPC/POPC- d_{31} MLVs significantly compared to the control sample lacking peptide (Figure 3A). The spectral widths decreased upon addition of the APH peptide, indicating an increase in the lipid bilayer fluidity caused by perturbation of lipid acyl chains upon peptide incorporation. As the concentration of APH peptide increased there was a loss of resolution of the spectrum as demonstrated by the disappearance of sharp edges of the peaks, also revealing an interaction between the APH peptide and the acyl chains of the lipid bilayer. In addition, this interaction increased the motion of the lipids and/or the dynamics of the vesicles, as evidenced by the increased isotropic peak intensity induced by higher amounts of APH peptide. Similar to the presence of an isotropic peak in the ^{31}P NMR measurements, the increased dynamics of the vesicles could be explained by the formation of small, rapidly tumbling vesicles [31]. The spectral line shape and isotropic component was even more pronounced when Hcf106 APH peptides were added to POPC/POPC- d_{31} /MGDG MLVs (Figure 3B). By contrast the line shape widths of the MLVs (either POPC/POPC- d_{31} or POPC/POPC- d_{31} /MGDG) were not changed from those of the control vesicles when in the presence of the control peptide (Supplemental Figure 2), although the presence of the control peptide did cause the formation of an isotropic peak. Taken with the static ^{31}P NMR data, these data indicate that

the Hcf106 peptide interacts with the POPC/MGDG vesicles, and does so in the same way as it interacts with the POPC vesicles.

However the changes in CSA line widths and in the ^2H NMR spectral line shapes due to the presence of the peptide were not very large (barely 10% change) and may be a reflection of how the peptide can engage the membrane. In vesicles containing the thylakoid-specific lipid, the APH peptide may be a membrane breaching peptide at high enough concentrations, similar to antimicrobial peptides, and this is evidenced by the increasing presence of the isotropic peak with increasing peptide amount. The presence of the MGDG lipid may enhance the membrane breaching capabilities of the APH peptide due to its tendency to not form a lamellar bilayer but rather to form a nonlamellar, inverted Hexagonal (Hexagonal II) phase.

All ^2H NMR powder pattern spectra were dePaked to probe the dynamic changes of the acyl chains upon Hcf106 APH peptide incorporation. S_{CD} order parameters are sensitive to intermolecular, intramolecular and collective motions, reflecting the orientation and dynamic change of the C-D bond vector [37, 38]. The smoothed segmental C-D bond order parameters (S_{CD}) were calculated from the dePaked spectra and plotted as a function of acyl chain carbon number for the different APH concentration at 25°C (Figure 4). As distance from the glycerol backbone increased, there was a decrease of S_{CD} for all samples, indicating increased motion and flexibility for the central and terminal acyl chain regions as compared to the proximal headgroup region (Figure 4A, filled circles). In the presence of 2 mol%, 4 mol% and 6 mol% APH peptides, the acyl chains of POPC lipid bilayers were more mobile compared to the control vesicles lacking peptide or vesicles containing the control peptide, as revealed by a decrease in the order of each C-D bond (Figure 4A). In the presence of the APH peptide, there was a larger decrease in the S_{CD} for the first three or four carbons from the ester than at the methyl end of the acyl chains (Figure 4A). Combined with the ^{31}P NMR data, these data suggest that Hcf106 APH peptide mostly sits on the membrane surface or may slightly tilt into the hydrophobic region of the phospholipid bilayers to interact with acyl chains in close proximity to glycerol backbones. It is also possible that Hcf106 APH peptide attached to the membrane surface by its hydrophilic face and induced a relatively compact environment on the membrane surface and more dynamic space in the hydrophobic region of the lipids. Further experiments are needed to verify these explanations. The S_{CD} profile for POPC/MGDG MLVs (Figure 4B) resembles that in POPC MLVs, except that POPC MLVs demonstrate a slightly larger S_{CD} and less perturbation caused by the incorporation of Hcf106 APH peptide.

4. Conclusion

This solid-state NMR study clearly shows lipid-peptide interaction. ^{31}P and ^2H solid-state NMR static spectra lineshape, CSA widths, and ^2H order parameters are utilized to investigate lipid-peptide interaction and the results are complimentary to each other. These data indicate that Hcf106 APH peptide interacts with the head group and acyl chains of phospholipid bilayers. The manipulation of the lipid composition clearly alters the line shape of the spectra, and the peptides interact more intensely with the thylakoid membrane-mimicking environment (POPC/MGDG MLVs). These data show for the first time that Hcf106 APH is by itself a membrane associated peptide, and also imply how the peptide could arrange itself to interact with lipids when incorporated into membrane systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Twin arginine transport protein Hcf106 contains an amphipathic α -helix (APH)
- Interaction of Hcf106 APH peptide and phospholipids was studied by Solid-state NMR
- The predicted APH of Hcf106 interacts with vesicle membranes

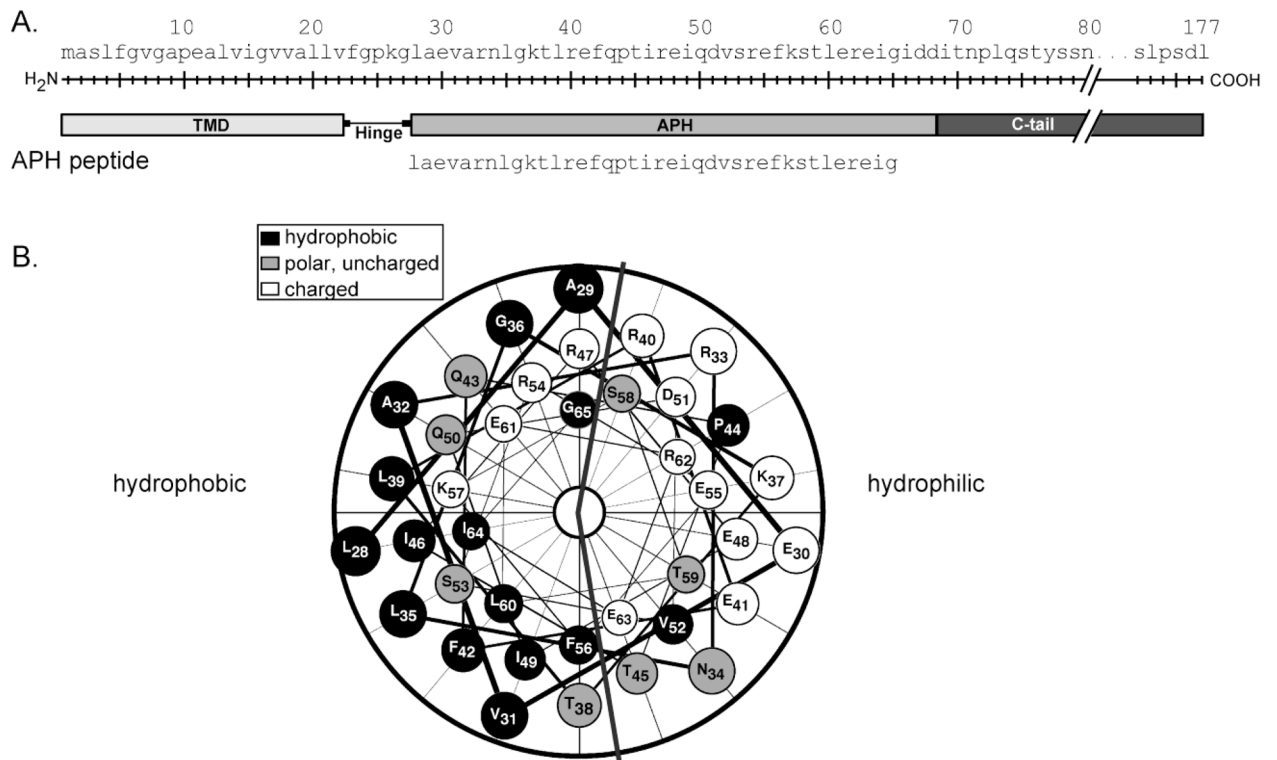


Figure 1. Schematic of Hcf106 and peptide used in this study

Peptides were synthesized on a CEM microwave peptide synthesizer (Matthews, NC) using Fmoc solid-phase peptide synthesis chemistry as described in the Materials and Methods.

(A) A schematic of Hcf106 showing the different domains. The amino acid sequence of the peptide representing the APH is indicated. (B) Helical wheel plot (DNASTar, Madison, WI) of the amino acids of the Hcf106 APH peptide indicating the hydrophilic and hydrophobic faces.

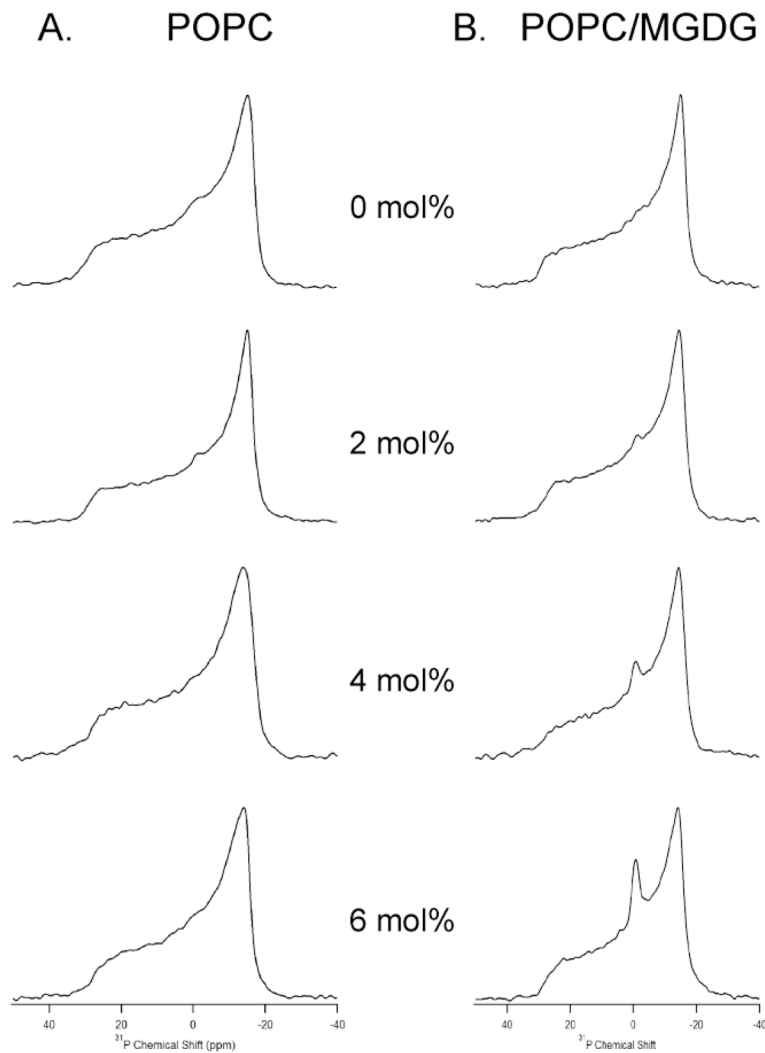


Figure 2. Hcf106 APH preferentially perturbs POPC/MGDG MLVs

(A) ^{31}P spectra of MLV samples showing the effect of Hcf106 APH on ^{31}P spectra line shape of POPC bilayers as a function of Hcf106 APH peptide concentration. (B) ^{31}P spectra of MLV samples showing the effect of Hcf106 APH on ^{31}P spectra line shape of POPC/ MGDG bilayers (85%:15%) as a function of Hcf106 APH concentration at 25°C. MLV samples of different lipids in the absence of Hcf106 APH, and in the presence of 2 mol %, 4 mol %, and 6 mol % Hcf106 APH with respect to lipids were investigated.

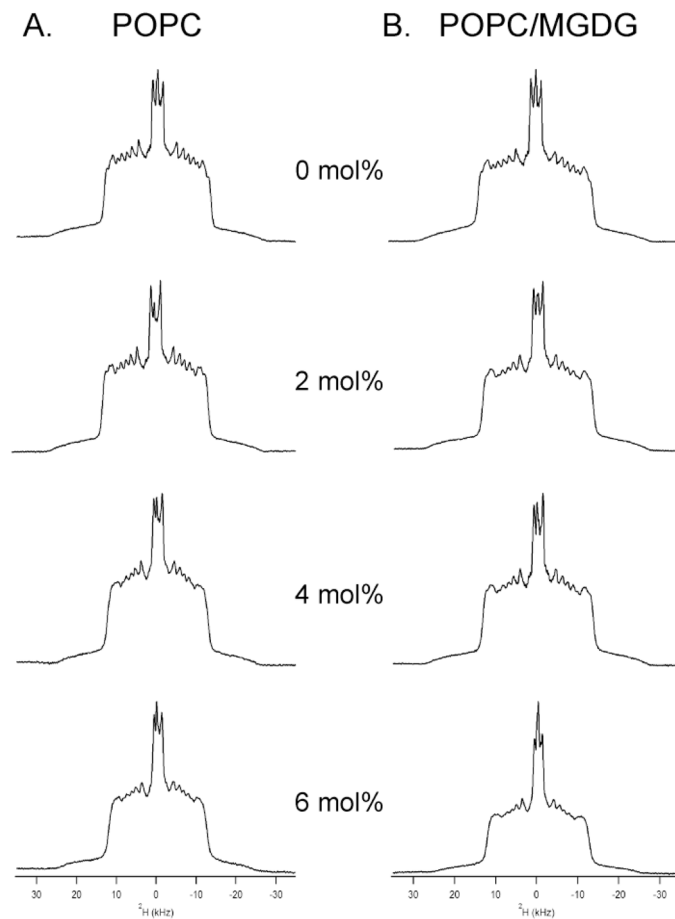
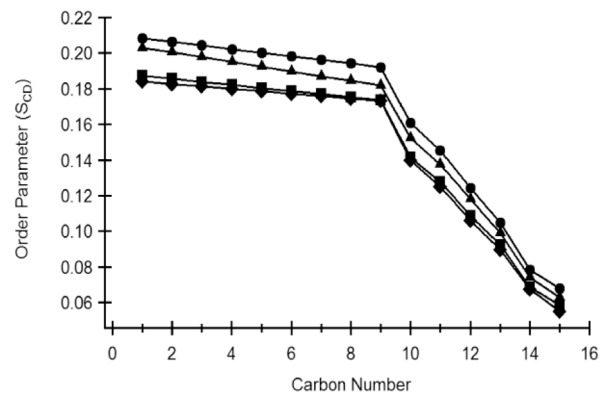


Figure 3. Hcf106 APH interacts with the bilayer of POPC or POPC/MGDG MLVs
 ^2H NMR spectra of MLV samples showing the perturbation of Hcf106 APH on lipids acyl chain of (A) POPC bilayers, and (B) POPC/MGDG bilayers as a function of Hcf106 APH concentration at 25°C. 5mg POPC- d_{31} phospholipids were used as deuterium probe. MLV samples of different lipids in the absence of Hcf106 APH, and in the presence of 2 mol %, 4 mol %, and 6 mol % Hcf106 APH with respect to lipids were investigated.

A. POPC MLVs



B. POPC/MGDG MLVs

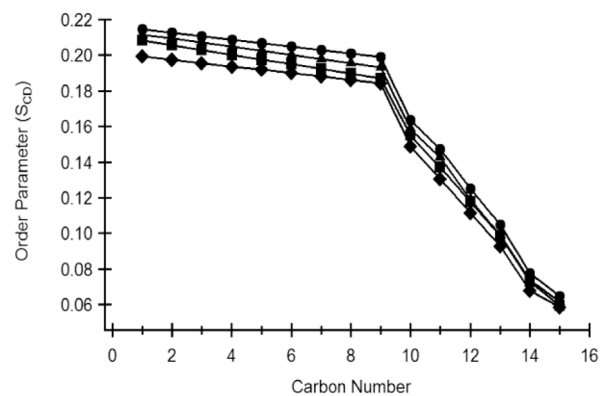


Figure 4. Hcf106 APH preferentially disturbs the lipid head group/acyl chain interface
Smoothed acyl chains orientational order S_{CD} profiles calculated by dePaking ^2H NMR spectra of Figure 3. (A) POPC MLVs, and (B) POPC/MGDG MLVs with and without Hcf106 APH at various concentrations. S_{CD} profiles calculated in the absence (●) or presence of Hcf106 APH at 2 mol % (▲), 4 mol % (■) and 6 mol % (◆), respectively.