Solid-State NMR Studies of the Structure, Dynamics, and Assembly of β-Sheet Membrane Peptides and α-Helical Membrane Proteins with Antibiotic Activities

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ABSTRACT
β-Sheet antimicrobial peptides and α-helical channel-forming colicins are bactericidal molecules that target the lipid membranes of sensitive cells. Understanding the mechanisms of action of these proteins requires knowledge of their three-dimensional structure in the lipid bilayer. Solid-state NMR has been used to determine the conformation, orientation, depth of insertion, oligomerization, mobility, and lipid interaction of these membrane peptides and proteins. We review the NMR methods developed and applied to study the structure and dynamics of these antibiotic membrane proteins. These studies shed light on how these peptides disrupt lipid membranes and provide fundamental insights into the folding of β-sheet and α-helical membrane proteins.

Introduction

A number of cationic peptides have been discovered in a wide range of organisms to have potent antibacterial, antifungal, and antiviral activities.[1,2] Their most common mode of action is to permeabilize the microbial cell membrane, depolarizing the cell. D-Enantiomers of these peptides show similar activities as their L-counterparts, indicating that the targets of these peptides are the achiral lipids of the membrane rather than chiral protein receptors inside the membrane or the cell.[3] In vitro, these peptides permeabilize lipid vesicles and form ion channels across planar lipid bilayers,[4] further indicating that their target is the lipid bilayer. The potency of these peptides and the lack of resistance that they encounter make them promising antibiotics. However, how these peptides disrupt the membrane structure and how they achieve this selectively against microbial but not eukaryotic membranes is not well understood. Several models have emerged to explain antimicrobial action: the transmembrane pore ("barrel stave") model, the in-plane ("carpet") model, and the toroidal pore model.[5,6] Determining the mechanisms of action of antimicrobial peptides requires both the high-resolution structure of the peptides and the morphology and dynamics of the lipid membranes.

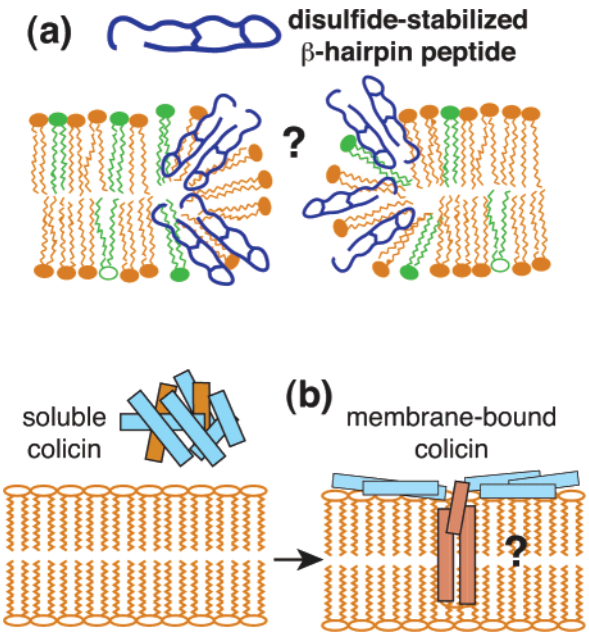

FIGURE 1. (a) Disulfide-stabilized β-hairpin antimicrobial peptides disrupt microbial cell membranes through mechanisms that are not well understood. The toroidal pore mechanism is illustrated here. (b) α-Helical channel-forming colicins spontaneously insert into lipid bilayers after undergoing large conformational changes. The membrane-bound structure is largely unknown.

In addition to their biological significance, many antimicrobial peptides adopt a disulfide-stabilized antiparallel β-strand conformation (Figure 1a) that makes them interesting targets for fundamental biophysical studies. The β-hairpins can be open-chain molecules such as tachyplesins and protegrins or cyclic molecules such as rhesus θ-defensin (RTD).[7,8] The structural stability and the small size of these molecules make them ideal systems for studying how β-sheet peptides insert into and assemble in the lipid bilayer.

Functionally similar to but structurally distinct from antimicrobial peptides are a family of large (60−70 kDa) α-helical bacteriocidal proteins called colicins. Channel-forming colicins kill bacteria by forming voltage-gated ion channels that deplete the membrane potential of the cell.[9] They enter the cell through receptors in the outer membrane, translocate across the periplasmic space, and then spontaneously insert into the cytoplasmic membrane and form voltage-dependent ion channels. The insertion step requires a large inside-out conformational change of the channel domain to adapt to the hydrophobic membrane (Figure 1b). Elucidation of the membrane-bound structure of the colicin channel domain is thus important for understanding membrane protein folding.

We have used solid-state NMR spectroscopy[10] to obtain detailed information on the dynamic structure of the β-sheet antimicrobial peptides and the α-helical colicins.

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Compared to other spectroscopic methods, solid-state NMR requires no bulky or mobile extrinsic probes and can be applied to proteins bound to lipid bilayers rather than to membrane-mimetic detergents. Thus, the structural information from solid-state NMR is the least perturbing and the most direct about the native state of membrane proteins. Here we review the solid-state NMR techniques that have been developed and applied to antimicrobial peptides and the channel domain of colicin Ia. We can now determine the protein orientation and depth of insertion with high resolution, obtain detailed information on protein mobility and protein−lipid interactions, and determine the oligomeric number of membrane peptides, which opens the possibility of studying membrane protein assembly.

Protein Conformation in the Membrane

To understand membrane protein conformational changes, one can directly measure (ϕ, ψ) torsion angles. Many solid-state NMR techniques have been developed for this purpose.[11] They either correlate the orientations of two bonds flanking the torsional bond of interest or measure angle-dependent internuclear distances. These techniques are usually applied to samples under magic-angle spinning (MAS) to give site-resolved spectra. For large membrane proteins such as colicin, two challenges arise in applying these techniques: single-residue resolution and sensitivity. For a typical NMR sample containing submicromoles of protein diluted in lipids, achieving single site sensitivity requires days of measuring time. Thus, experiments that determine (ϕ, ψ) angles with the fewest frequency dimensions and the highest site resolution are desirable. Two complementary techniques have been developed for this purpose. The first selectively detects the signals of α-helical residues by exploiting their relatively small C^α chemical shift anisotropies (CSAs):[12] a rotor-synchronized π-pulse train dephases the C^α signals according to their CSAs and is inserted into a 2D $^{15}N-^{13}C$ correlation experiment. The resulting 2D spectra retain the helix resonances while suppressing the sheet signals.[13] This α-helix selection experiment was demonstrated on ubiquitin and applied to colicin Ia channel domain. Comparison of the C^α CSAs and the isotropic shifts between the soluble and membrane-bound states showed a small increase in helicity for the membrane-bound protein.[14] Most likely, this results from a change of helical residues' torsion angles to more ideal values due to the reduced packing constraints in the open conformation of the membrane-bound colicin compared to the soluble protein.[14]

The second conformation-determination technique selects β-sheet signals over α-helical ones based on their different ϕ torsion angles.[15,16] β-sheet residues have nearly antiparallel N−H and $C^\alpha-H^\alpha$ bonds ($\phi \approx -120°$), while helical residues have staggered bonds ($\phi \approx -60°$). As a result, the β-sheet C^α signals decay under the sum and difference N−H and $C^\alpha-H^\alpha$ dipolar couplings more slowly than the helix signals. In the middle of a MAS rotor period, the difference is maximal with the helix intensities nearly zero while the sheet signals are still present. With measurement of the ϕ-dependent dipolar coupling only at the center of the rotor period rather than throughout, the experiment is more efficient, thus allowing the inclusion of 2D $^{15}N-^{13}C$ correlation to resolve multiple resonances. Applied to colicin Ia channel domain, the experiment indicated a slightly reduced β-sheet content in the membrane-bound case (unpublished result), consistent with the α-helix selection experiment.

Protein Orientation in the Membrane

An important aspect of membrane protein structure is its orientation relative to the lipid bilayer. Whether an α-helix or β-sheet is transmembrane or surface-associated reveals the mechanism of action of the peptides. The inherent anisotropic nature of nuclear spin interactions allows the accurate determination of membrane protein orientations, since NMR frequencies reflect the orientation of the molecule-fixed nuclear spin tensor relative to the magnetic field, $\mathbf{B}_0$.

A protein bound to unoriented liposomes is randomly oriented with respect to $\mathbf{B}_0$, even though its orientation in the bilayer is unique. This yields a frequency distribution $\omega = {}^1/_2\delta(3\cos^2\theta - 1 - \eta\sin^2\theta\cos 2\phi)$, where δ and η are the anisotropy and asymmetry parameters of the interaction tensor and θ and ϕ are the polar and azimuthal angles of $\mathbf{B}_0$ in the principal axis system of the tensor. The resulting powder spectrum makes it impossible to extract the protein orientation in the bilayer. To retrieve this information, the lipid bilayers must be uniaxially oriented so that all peptides have the same orientation and thus a single frequency. Usually the bilayer normals are aligned parallel to $\mathbf{B}_0$, so the peptide orientation relative to $\mathbf{B}_0$ is the same as its orientation to the bilayer normal. Uniaxial alignment of lipid bilayers can be achieved mechanically using thin glass plates or magnetically using bicelles.[17]

Two spin interactions, the ^{15}N chemical shift and $^{15}N-^1H$ dipolar coupling, are commonly used to determine the orientation of α-helical peptides, since these tensors are approximately parallel to the helical axis and thus reflect the helix tilt. The amide ^{15}N has a δ of ∼150 ppm and a line width of 3−5 ppm in uniaxially aligned samples. Thus, the angular resolution of the ^{15}N chemical shift spectrum is high. One can further enhance both the site resolution and the angular resolution of orientation determination by correlating the ^{15}N chemical shift with the $^{15}N-^1H$ dipolar coupling. Due to the slight nonlinearity among the ^{15}N chemical shift z-axis, the N−H bond, and the helical axis, the 2D spectra exhibit wheel-like patterns whose size and frequency are exquisitely sensitive to the tilt angle.[18,19]

For β-sheet peptides, ^{15}N chemical shift is less favorable for orientation determination. Since the N−H bonds are nearly perpendicular to the strand axis, both transmembrane and in-plane β-sheets have their N−H bonds roughly perpendicular to the bilayer normal and thus are less distinguishable. Two approaches were developed to break this degeneracy. The first utilizes the carbonyl

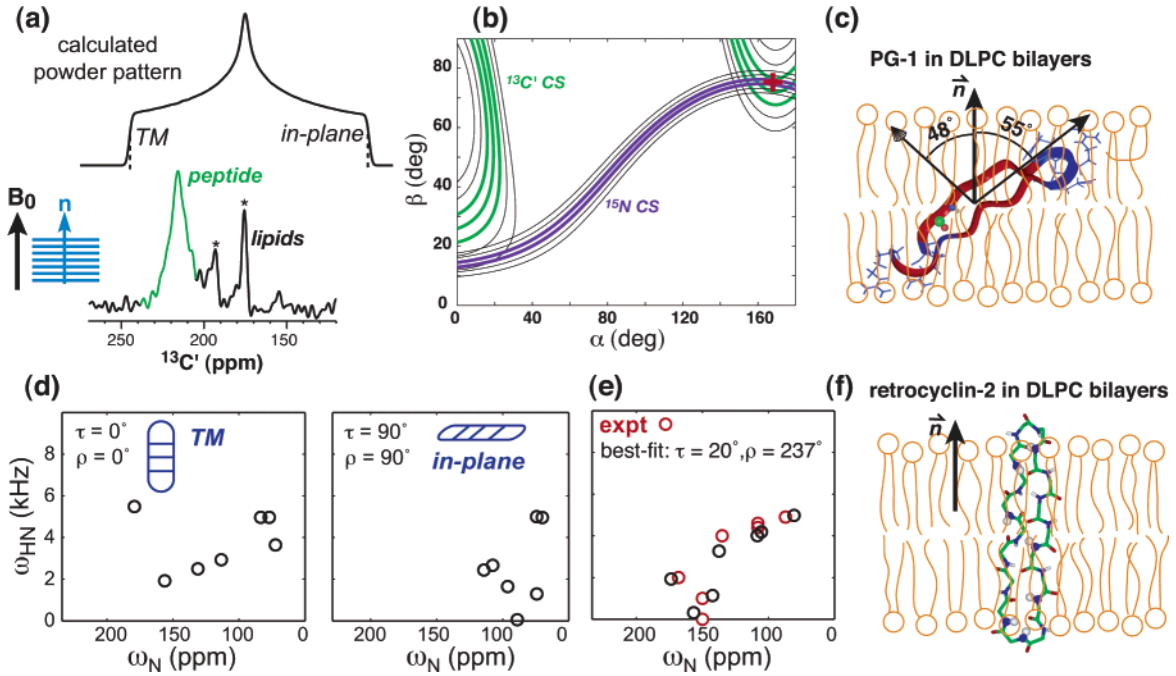

FIGURE 2. (a) Calculated $^{13}C'$ chemical shift powder pattern and experimental $^{13}C'$ spectrum of uniaxially aligned PG-1 in DLPC bilayers. (b) The $^{13}C'$ and ^{15}N chemical shifts of oriented PG-1 restrain the peptide orientation to a unique set of polar coordinates (β, α). (c) These (β, α) angles correspond to a tilted PG-1 orientation. (d) Calculated 2D $^{15}N-^{1}H/^{15}N$ correlation spectra of transmembrane (TM) and in-plane β-hairpins. (e) The 2D spectrum of retrocyclin-2 in DLPC bilayers. Best-fit yields a transmembrane orientation (f) with a tilt angle of 20° and a rotation angle of 237°.

chemical shift interaction, which better reflects the β-sheet geometry: the x-axis of the C′ CSA tensor points along the strand axis, while the z-axis is perpendicular to the β-sheet plane. Combining $^{13}C'$ and ^{15}N chemical shifts, we measured the orientation of the β-hairpin peptide protegrin-1 (PG-1) in DLPC bilayers and found the strand axis to be tilted from the bilayer normal by ~55° (Figure 2a–c).[20] Alternatively, one can utilize 2D N–H dipolar and ^{15}N chemical shift correlation spectroscopy to obtain characteristic patterns that are sensitive to both the tilt angle of the strand axis and the rotation angle of the sheet plane. In these 2D spectra, the turn residues give rise to outlier peaks distinct from the strand signals, further facilitating the orientation determination. Simulated 2D spectra for a transmembrane and in-plane β-hairpin (Figure 2d,e) show that these are readily distinguishable. Using this approach, we found that retrocyclin-2, a cyclic β-hairpin peptide, is transmembrane in DLPC bilayers.[21]

While the amino acid sequence plays a crucial role in dictating membrane peptide orientation, environmental factors such as the hydrophobic mismatch between the peptide and the lipids can also affect the orientation. For example, retrocyclin-2 is transmembrane in DLPC bilayers but tilted to the plane in POPC bilayers.[21] PG-1 is tilted in DLPC bilayers but disordered in POPC bilayers.[20] The peptide concentration also affects its orientation: increasing the concentration changes the orientation of magainin, protegrin, and RTD-1 from in-plane to transmembrane.[22] Thus, the stable orientation of membrane peptides depends on multiple factors.

Depth of Insertion

The depth of insertion is a valuable alternative probe of membrane protein topology, since orientation determination is often limited by the practical difficulty of aligning the membrane. We developed two MAS techniques to determine the depth of insertion of membrane proteins. The first experiment introduces paramagnetic Mn^{2+} ions to the membrane surface to enhance the T_2 relaxation rates of nuclear spins in a distance-dependent fashion.[23,24] Since the relaxation enhancement also depends on the peptide motional correlation time and the Mn^{2+} residence time, which are difficult to measure, one can calibrate the insertion depth of the peptide with that of the lipid by comparing the relaxation enhancement of the two. The depths of lipid segments are well-known from diffraction studies (Figure 3a).[25]

Using Mn^{2+}-bound DLPC bilayers, we found that PG-1 is completely inserted into the membrane with the two ends of the β-hairpin (residues F12 and G2) experiencing the largest relaxation enhancement and thus being closest to the membrane surface, while the middle of the β-strands (L5 and V16) show relaxation enhancement comparable to the lipid acyl chains [24] (Figure 3b). This is consistent with the orientation of PG-1 obtained from ^{13}C and ^{15}N chemical shifts.[20] Moreover, a quantitative mismatch between the bilayer thickness and the PG-1 length suggests a local thinning of the DLPC bilayer by 8–10 Å.[24]

The second approach for determining the immersion depth of membrane proteins is ^{1}H spin diffusion between lipids and the protein or between water and the protein.

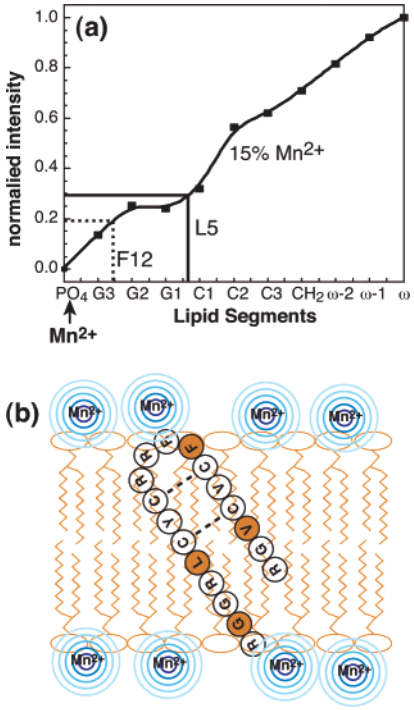

FIGURE 3. (a) Mn^{2+}-induced T_2 relaxation enhancement decreases signal intensities according to the nuclear spin distance from the Mn^{2+} ions. In PG-1, the F12 signal is reduced similarly to the lipid glycerol signals while the L5 reduction is similar to the acyl chain C1. (b) Schematics showing that PG-1 is inserted into DLPC bilayers in a tilted fashion. F12 and G2 are immersed in the glycerol backbone region, while L5 and V16 are sequestered in the hydrophobic chain region.[24]

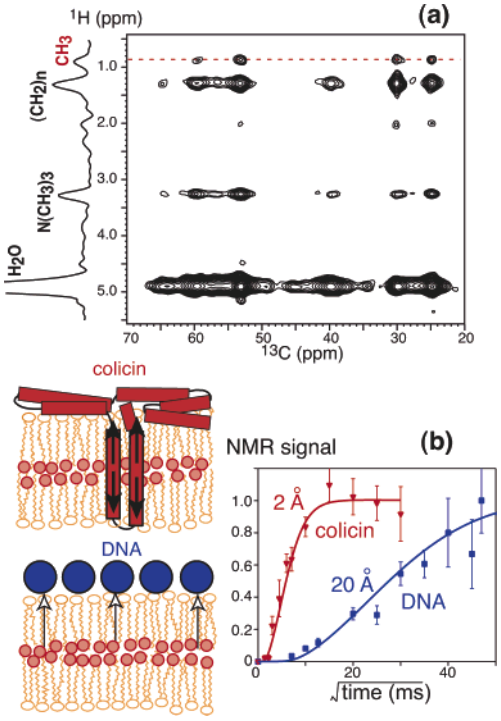

FIGURE 4. (a) Representative 2D $^1H-^{13}C$ correlation spectrum of membrane-bound colicin, highlighting the lipid methyl−protein cross-peaks (dashed line). (b) Magnetization buildup curves from the methyl protons to colicin (red) and DNA (blue). The buildup rates indicate that colicin has a transmembrane domain while DNA resides purely on the membrane surface.[28]

The transfer of magnetization from a source proton to a sink proton is mediated by distance-dependent dipolar couplings. From the intensity buildup of the sink proton as a function of spin diffusion time, we can obtain the protein−lipid or protein−water distance. When the interbilayer water protons act as the magnetization source, the intensity buildup reveals the depth of the protein from the membrane surface. To select the water ^1H signal to initiate spin diffusion, one can freeze the lipids while leaving the interbilayer water partly mobile. This low-temperature experiment can be conducted in one dimension with detection of the protein ^{13}C or ^{15}N signals. With this method, qualitative depth information was obtained on colicin E1 channel domain, indicating that the protein is mainly associated with the membrane surface.[26] More quantitative depths can also be determined, as demonstrated on gramicidin A.[27]

When the lipid chain methyl protons are used as the magnetization source, the distance of the protein from the bilayer center can be determined. This experiment is particularly useful when a small portion of a large protein is deeply inserted, which is difficult to detect with the water-initiated experiment. The methyl ^1H signal can be selected not by freezing (since the methyl group mobility is comparable to the rest of the lipid) but by chemical shift, which is readily achievable by a 2D $^1H-^{13}C$ correlation experiment (Figure 4a). However, without freezing, the diffusion coefficient of the liquid-crystalline lipids is much smaller than that of the rigid protein, thus the intensity buildup curve mainly reflects the shortest lipid−protein distance rather than a specific residue's distance to the membrane center. Despite this loss of site specificity, the 2D experiment is valuable since the structural information often cannot be obtained any other way.

The 2D spin diffusion experiment was used to determine the topology of membrane-bound colicin Ia channel domain.[28] The methyl ^1H buildup curve indicates that the protein contains a small fraction of residues within 2−4 Å of the bilayer center. In comparison, the water−protein cross-peaks are much higher, indicating that most residues reside on the membrane surface. Thus, an umbrella model describes the colicin topology well.[29] In contrast, in a cationic lipid−DNA complex, the methyl−DNA buildup curve is an order of magnitude slower than that of colicin and is consistent with a distance of ~23 Å (Figure 4b). Thus, the anionic DNA remains on the surface of the cationic bilayer, supporting a lamellar structure of the complex.[30]

The 2D ^1H spin diffusion experiment was also used to determine the depth of insertion of PG-1 in POPC bilayers. PG-1 is close to both the headgroup and the chain ends of the lipid and thus is well inserted. This information could not be obtained from orientation measurements due to the large disorder of the POPC/PG-1 membrane.[20]

Peptide Oligomerization in Lipid Bilayers

Oligomerization of membrane peptides is an important step in the folding of polytopic membrane proteins.[31,32]

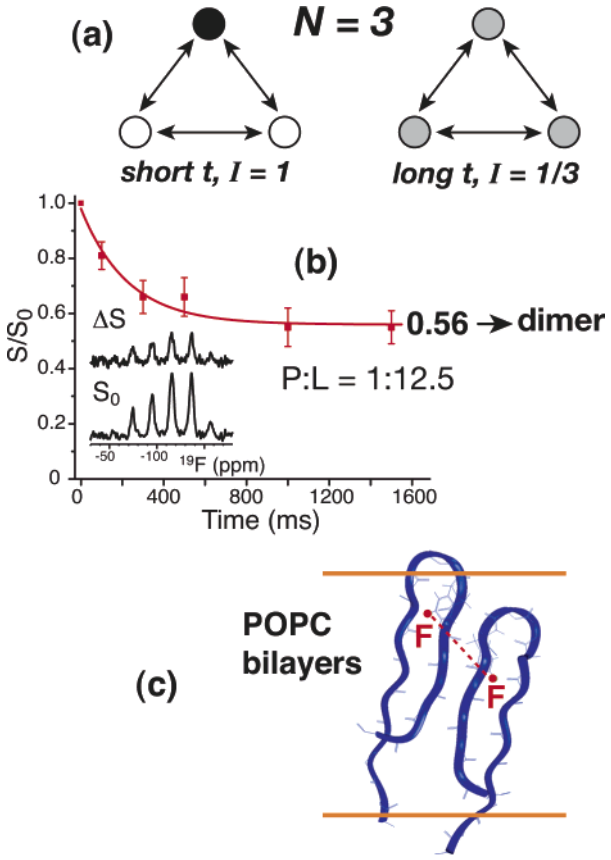

FIGURE 5. (a) Principle of ^{19}F spin diffusion for determining the oligomeric number of membrane peptides. Spin diffusion among N orientationally different molecules reduces a stimulated echo to $1/N$. (b) ^{19}F spin diffusion curve of PG-1 in POPC bilayers. The equilibrium value indicates dimer formation. (c) The PG-1 dimer is parallel with the C-strand lining the dimer interface.

For β-sheet membrane peptides, oligomerization is important for sequestering the polar backbone N−H and C=O groups in the hydrophobic bilayer. For β-hairpin peptides, about half of the polar groups are hydrogen-bonded intramolecularly while the remaining ones would be exposed to the bilayer unless the peptide is oligomerized. The existence of such oligomeric structures was rarely proven experimentally but commonly assumed in mechanistic models of antimicrobial action.[4,33] The paucity of structural information on membrane peptide oligomerization mainly results from the lack of suitable techniques. Analytical ultracentrifugation and gel electrophoresis are applicable only to peptides bound to detergent micelles.[32]

We developed a ^{19}F spin diffusion NMR technique to determine the oligomeric number of membrane peptides in lipid bilayers.[34,35] The experiment transfers ^{19}F magnetization between orientationally different molecules. The orientation difference changes the ^{19}F chemical shift frequency, reducing the intensity of a stimulated echo. At equilibrium, the echo intensity is $1/N$ of the initial value, where N is the number of orientationally unique molecules. Thus, a dimer gives an equilibrium intensity of $^1/_2$, a trimer $^1/_3$, and so on (Figure 5a). The use of ^{19}F spin is advantageous due to its strong dipolar coupling and its large CSA, which makes the experiment sensitive to long distances and small orientational differences. This method is able to detect $^{19}F-^{19}F$ distances up to 15 Å.[34]

Using this ^{19}F spin diffusion method, we found that PG-1 associates as dimers in POPC membranes at high peptide concentrations (Figure 5b).[34] Decreasing the concentration reduced the dimer fraction. From the equilibrium monomer−dimer ratio, the Gibbs free energy of dimerization was estimated to be −10.2 kJ/mol, in good agreement with the ΔG of hydrogen bond formation in β-sheet peptides.[36] Further experiments indicated that the β-hairpins pack in a parallel fashion with the C-terminal strands lining the dimer interface (Figure 5c).[37]

Protein Dynamics in the Membrane

Protein mobility gives important insights into protein function. Solid-state NMR is an excellent method for investigating molecular motions on time scales from nanoseconds to seconds. Motions faster than the nuclear spin interactions can be studied from dipolar line-narrowing experiments. For example, the one-bond C−H dipolar coupling is 22.7 kHz in the absence of motion but reduced in the presence of fast motions. The motionally averaged couplings can be measured using 2D dipolar-shift correlation experiments, which resolve the couplings of different sites by ^{13}C isotropic shifts. The coupling reduction from the rigid-limit value gives the bond order parameter, $S_{CH} = \omega_{CH}/\omega_{CH}$, which reflects the amplitude of motion. For small-amplitude axially symmetric motions, the order parameter depends on the root-mean-square angle fluctuation, $\sqrt{\langle\theta_{CH}^2\rangle}$, as $S_{CH} = 1 - {}^3/_2\langle\theta_{CH}^2\rangle$.[38] Similar 2D correlation schemes also yield semiquantitative $^1H-^1H$ dipolar couplings. For rigid solids, the $^1H-^1H$ couplings are ~50 kHz for CH groups and ~60 kHz for CH_2 groups. Reduction from these values signifies motion. In addition to motional amplitudes, motional geometry can also be obtained from spectral line shapes. For example, methyl group rotation and aromatic ring flips give characteristic line shapes in the indirect dimension of a 2D Lee−Goldburg cross-polarization (LG-CP) spectrum.[39]

Using the line shape measurements, we found that membrane-bound colicin Ia channel domain exhibits smaller couplings than the soluble protein,[40] indicating that colicin undergoes larger-amplitude motions in the membrane due to extensive contact with the lipids. This implies an open topology of the membrane-bound protein, which differs from the compact globular shape of the soluble colicin. Further dipolar coupling measurements as a function of the anionic lipid content and salt concentration indicate that the membrane-bound colicin is more rigid under high salt and low anionic lipid content than under low salt and high anionic lipid content. The former gives the more physiological membrane surface potential, which suggests that colicin channel activity requires sufficient segmental rigidity to allow the helices to undergo cooperative conformational changes, which are necessary for translocating the channel-forming helices across the bilayer.[41] For PG-1, mobility depends

on the membrane thickness. The peptide rotates uniaxially around the bilayer normal in DLPC bilayers but is immobilized in POPC bilayers,[42] which correlates with its selective disruption of POPC bilayers but not DLPC bilayers.[20]

Chemical shift anisotropy can also be used to extract motional information. However, since most rigid-limit CSA tensors are not uniaxial, it is generally not possible to extract a single order parameter, and a full order tensor analysis is more appropriate.[43] But when the motionally averaged CSA is uniaxial for multiple residues in the peptide, then the peptide most likely undergoes rigid-body rotation around the bilayer normal. This uniaxial motion was observed in ovispirin, an α-helical antimicrobial peptide,[44] and PG-1.[42] Given the typical membrane viscosity of 1−10 P, rigid-body rotation of small peptides is not surprising.[45] In fact, the lack of such rotation suggests either oligomerization or confinement of the peptide in membrane defects. The immobilization of PG-1 dimers in POPC bilayers strongly suggests that the peptide is trapped in membrane defects such as toroidal pores.[42]

Slow motions on the millisecond time scale or longer can be studied by exchange NMR, which measures an anisotropic frequency before and after a mixing period during which molecular reorientation occurs. The reorientation changes the frequency, resulting in nondiagonal peaks in 2D spectra or reduced intensities in 1D stimulated echoes.[35,46] The mixing-time dependence of the spectra gives the correlation time of motion, while the 2D cross-peak patterns yield the reorientation angle distribution. Using exchange NMR, we found that PG-1 dimers undergo slow motions with a correlation time of 0.7 s in POPC bilayers[42] while colicin Ia channel domain is immobilized on this time scale.[40] An interesting application of exchange NMR is the lateral diffusion of peptides over the curved surface of lipid vesicles. For micrometer-sized lipid vesicles, this lateral diffusion produces rotations in tens of milliseconds, detectable by exchange NMR. Curvature changes of the vesicles manifest as changes in the 2D cross-peaks or 1D stimulated-echo intensities. We found that PG-1 maintains the radius of curvature of DLPC vesicles but reduces that of POPC vesicles by a factor of 3, indicating vesicle fragmentation due to membrane rupture.[46]

Protein−Lipid Interactions

The selective destruction of microbial cells but not eukaryotic cells by antimicrobial peptides indicates that the peptide−lipid interaction depends on the membrane composition. Microbial membranes are rich in anionic lipids but poor in cholesterol, while the opposite is true for eukaryotic cell membranes. ^{31}P and ^{2}H NMR are excellent probes of peptide−lipid interactions. ^{31}P chemical shift is sensitive to the lipid phase, headgroup conformation, and membrane surface perturbations, while ^{2}H quadrupolar couplings of chain-deuterated lipids probe the dynamics of the hydrophobic part of the membrane. To spectrally resolve different lipid morphologies induced by the peptides, one can utilize uniaxially aligned membranes. The peptide−lipid interactions of PG-1, RTD-1, and retrocyclin-2 have been investigated as a function of membrane composition. All three peptides disrupt anionic membranes while largely maintaining the structural integrity of zwitterionic cholesterol-containing membranes.[20,21,47] However, the mode of membrane disruption differs. PG-1 causes the formation of isotropic vesicles in POPC/POPG bilayers, manifested as an isotropic peak in the ^{31}P spectra,[20,47] while RTD-1 induces the formation of micrometer-sized lipid cylinders, manifested as a symmetric ^{31}P line shape.[48]

Membrane disruption by antimicrobial peptides depends on the amino acid sequence. The removal of the disulfide bonds abolished the bilayer-perturbing activity of PG-1, while the reduction of the number of arginine residues merely attenuated the membrane disorder.[47] ^{1}H solution NMR indicates that the linear PG-1 is a random coil in solution while the charge-reduced PG-1 retains the β-hairpin fold. Thus, the β-hairpin conformation appears to be essential for maintaining the antimicrobial activity of PG-1.[47]

Membrane peptides can change the fluidity of the lipid bilayer. ^{2}H spectra of bilayers containing colicin Ia channel domain and ovispirin showed reduced quadrupolar couplings, indicating that the acyl chains become more dynamically disordered.[14,44] This increased fluidity indicates lateral expansion of the membrane, which is consistent with the in-plane orientations of these peptides. In contrast, PG-1 and RTD-1 do not affect the ^{2}H quadrupolar couplings,[20] consistent with their transmembrane orientation.[48]

Concluding Remarks

Much molecular insight has been gained from solid-state NMR investigations of the structure and dynamics of the α-helical colicin Ia channel domain and the β-sheet PG-1 and related peptides with antibiotic activities. Membrane-bound colicin Ia channel domain adopts an umbrella-like topology with a small portion inserted all the way to the hydrophobic center of the bilayer, while the majority remains on the membrane surface. Compared to the soluble state, the membrane-bound state is more flexible, consistent with its open topology, which allows extensive contacts with the lipids. This open topology differs fundamentally from the compact globular shape of the soluble colicin and is supported by ^{13}C spin diffusion data, which indicate a lengthening of interhelical distances upon membrane binding.[49] Under physiological membrane surface potentials, membrane-bound colicin Ia channel domain exhibits reduced segmental mobility, suggesting that optimal channel activity requires the helices to undergo cooperative conformational changes as rigid units to translocate key channel-forming helices across the bilayer.

The small disulfide-linked β-hairpin peptide PG-1 has very different structural properties that are membrane-dependent. In thin neutral lipid bilayers, PG-1 is trans-

membrane and significantly tilted and undergoes uniaxial rotation around the bilayer normal. In thick bilayers, the PG-1 β-hairpins form parallel dimers that are immobilized, possibly by membrane defects. PG-1 interaction with the lipid depends on the membrane composition: it fragments anionic bilayers into small isotropic vesicles while retaining the lamellar structure of cholesterol-containing bilayers. Disulfide bonds are essential to PG-1 action: membrane disruption is abolished without the disulfide bonds. PG-1 perturbs the membrane organization without affecting the fluidity of the hydrophobic region. This contrasts with the predominantly surface-associated colicin Ia channel domain, which laterally expands the bilayer, increasing the lipid chain mobility.

Therefore, solid-state NMR spectroscopy is capable of yielding comprehensive structural information on both small membrane peptides and large membrane proteins. The highest-resolution structural information is the peptide orientation and depth of insertion, and exquisite details of peptide dynamics and peptide−lipid interactions can also be obtained. The main remaining challenge is to determine the 3D structures of these membrane proteins with atomic resolution. This requires improved sample preparation methods to enhance the spectral resolution and higher magnetic field strengths and more efficient polarization transfer techniques to enhance the spectral sensitivity. The determination of the oligomeric structure of antimicrobial peptides will not only elucidate how these peptides rupture microbial cell membranes but also provide fundamental insight into the thermodynamics of β-sheet membrane protein folding. Similarly, the high-resolution structure of the membrane-bound channel domain of colicins will enhance our understanding of how α-helical proteins spontaneously insert into and fold in biological membranes.

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