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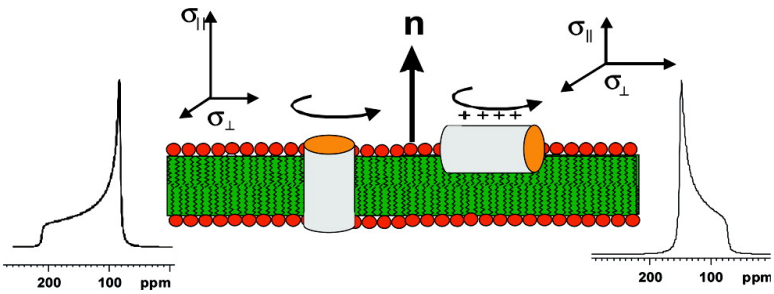

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The Membrane Alignment of Helical Peptides from Non-oriented ^{15}N Chemical Shift Solid-State NMR Spectroscopy

Lydia Prongidi-Fix, Philippe Bertani, and Burkhard Bechinger*

Institut de Chimie, Univeristé Louis Pasteur-CNRS UMR7177, 4 rue Blaise Pascal, 67000 Strasbourg, France

Received April 25, 2007; E-mail: bechinger@chimie.u-strasbg.fr

The analysis of the structure, topology, and dynamics of membrane polypeptides provides important clues for the understanding of their functional mechanisms. The interactions of these peptides and proteins are often characterized by a high degree of mobility, and solid-state NMR spectroscopy is a method that allows the monitoring not only of the structure but also of the dynamics of membrane polypeptides.[1−10] Deuterium and proton-decoupled ^{15}N solid-state NMR spectroscopy has proven particularly useful during the investigation of the topology of polypeptide domains.[11] In previous work it has been demonstrated that the ^{15}N chemical shift correlates with the alignment of the NH vector relative to the magnetic field direction and therefore also the tilt angle of helical polypeptides.[6] This is due to the favorable properties of the amide bond ^{15}N chemical-shift tensor, which is characterized by a unique σ_{33} principal axis value of 230 ppm and an alignment within 20° of the NH-bond.[12−15] Within an α-helical conformation σ_{33} is therefore oriented close to parallel to the helix long axis (Figure 1)

As a consequence, when reconstituted into membranes that are oriented with their normal parallel to the magnetic field direction, transmembrane peptides exhibit chemical shift values approaching σ_{33}, whereas peptides residing along the surface show resonances in the range of σ_{11} and σ_{22} (65 and 85 ppm, respectively).[6,16] Thus the ^{15}N chemical shift interaction has been used to determine helix alignments of peptides, mechanically oriented between glass plates or plastic sheets or when reconstituted into bicelles.[6,17,18]

Theoretical considerations indicate that for membrane-associated peptides or small proteins rotational diffusion around the normal of liquid crystalline bilayers is sufficiently fast to result in motional averaging of the chemical-shift tensor.[6,9] The spectral simulations also indicate that the shape and anisotropy of such symmetric chemical-shift tensors are a function of the axis of motional averaging and thereby an indicator of the relative helix alignment. Similar effects have been used in the past during the investigation of conformational changes in membrane lipids by ^{2}H or ^{31}P solid-state NMR,[19,20] studies that have been performed in non-oriented membrane samples. Although, theory would predict that the use of non-oriented membrane samples is a feasible approach for the study of the membrane topology of helical polypeptides using proton-decoupled ^{15}N solid-state NMR spectroscopy,[6] this concept has so far not been tested experimentally.

All peptides, including the transmembrane hΦ19W (KKKAL LALLA LAWAL ALLAL LAKKK) and the amphipathic helix KL14 (KKLLK KAKKL LKKL) were synthesized by solid-phase peptide synthesis.[21] At the underlined positions ^{15}N-labeled Fmoc-protected alanine or leucine analogues were incorporated. The α-helical model peptides KL14 and hΦ19W adopt stable in-plane and transmembrane alignments, respectively.[21] To allow for a direct comparison with previous studies, the membranes were prepared on a large continuous glass surface analogous to the preparations of oriented samples.[21] However, after equilibration at 95% relative humidity the material was scratched from the surface and transferred into sealed NMR tubes. Under these conditions the membrane hydration corresponds to about 15 water molecules per phosphatidylcholine.[22]

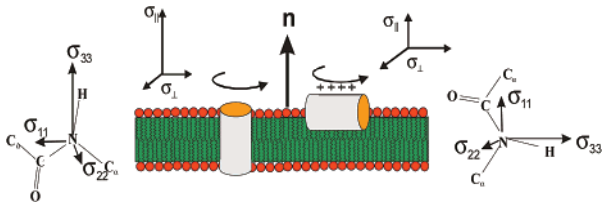

Figure 1. Figure 1 shows the ^{15}N amide tensor in the context of the peptide bond and its alignment relative to the membrane normal (**n**) when being part of a transmembrane (left) or an in-plane oriented helix (right). The averaged tensor is sketched next to the helices.

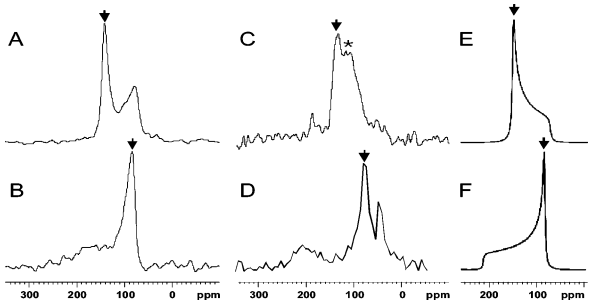

Figure 2. Proton decoupled ^{15}N chemical shift spectra of 50 mg each of [^{15}N-leucine-10]-KL14 (A), [^{15}N-leucine-15]-hΦ19W (B), [^{15}N-alanine-15]-magainin 2 (C), and [^{15}N-alanine-10]-Vpu$^{1-27}$ (D) when reconstituted into 400 mg of non-oriented DMPC. The molar lipid-to-peptide ratios are 20, 30, 33, and 33, respectively. The spectra were recorded at 310 K in a field of 9.4 T (A,B) or 11.8 T (C,D) using a ^{1}H pulse width of 8 μs, a spin lock of 0.8 ms using B_1 fields of 31 kHz (C,D) or 1.6 ms (A,B) and a recycle delay of 3 s. Typically, the number of scans is 80 000. Before Fourier transformation an exponential apodization function (LB 300 Hz) was applied. Simulations of ^{15}N chemical shift spectra exhibiting rotational averaging around the σ_{11} (E) or the σ_{33} axis of the static ^{15}N chemical shift tensor (F) are presented for comparison. The star indicates the isotropic chemical shift position; the arrows indicate the $\sigma_{\perp}$discontinuities. The signal intensity at 45 ppm is from DMPC.

The resulting proton-decoupled cross-polarization ^{15}N solid-state NMR spectra are shown in Figures 2A and B. At room temperature the peptides exhibit rotational diffusion around the bilayer normal that is fast when compared the time scale of the ^{15}N chemical shift interaction. Therefore, the experimental spectra exhibit discontinuities at positions similar to those calculated when rotational averaging around the σ_{11}/σ_{22} (Figure 2E) or the σ_{33} axis is taken into account (Figures 2F).

The data agree well with previous spectra where oriented membrane samples of these peptides were investigated with the normal parallel or perpendicular to the magnetic field direction,[11,21] The maximum and the edge of the shoulder observed in the powder pattern of KL14 (Figure 2A) corresponds to the signal intensities at 75 and 143 ppm, respectively, in DMPC oriented samples.[21] The

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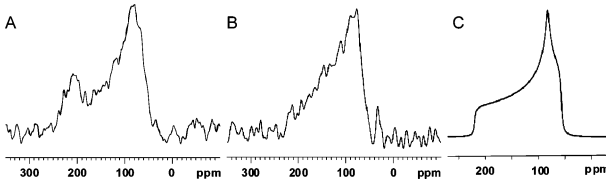

Figure 3. Proton decoupled ^{15}N chemical shift spectra of [^{15}N-leucine-10]-KL14 reconstituted into DMPC bilayers (T_c = 296 K) and recorded at 295 K (A) and 280 K (B). Spectrum C represents a simulated powder pattern line shape with σ_{11} = 65 ppm, σ_{22} = 85 ppm, and σ_{33} = 230 ppm.

corresponding intensities observed for hΦ19W in DOPC are 205 and 76 ppm, respectively.[21]

The distortions of the line shapes are due to alignment-dependent cross-polarization efficiencies most pronounced at orientations of the N−H dipole close to the magic angle (Figure 2A,D) and has been observed previously with other peptides[23] and lipids.[24] This effect gradually disappears when the mobility of KL14 is reduced by cooling the sample to temperatures less than or equal to the liquid crystalline-gel phase transition (Figure 3A,B). When compared to Figure 2A the motions in the gel phase are reduced and as a consequence the ^{15}N chemical shift anisotropy increased, thereby approaching the NMR line shape of a static powder pattern (Figure 3C).

The data shown in Figures 2 and 3 provide a proof of concept by indicating that, provided averaging around the membrane normal is fast, ^{15}N chemical shift line shapes are obtained that are characteristic for the orientation relative to the membrane normal of the helices carrying the ^{15}N label.

The proton-decoupled ^{15}N solid-state NMR spectrum of the antimicrobial peptide magainin 2 when associated with DMPC bilayers is shown in Figure 2C. The 26 residues peptide is strongly cationic and adopts random coil conformations in aqueous solution and an amphipathic α-helical structure when associated with membranes. When reconstituted into oriented membranes the ^{15}N chemical shift position at 84 ppm is indicative of an alignment parallel to the surface.[25] The powder pattern line shape shown in Figure 2C is indicative of a high degree of motional averaging around the membrane normal, and the $\sigma_\perp$ value at 135 ppm agrees well with the peptide's alignment along the surface. The additional isotropic signal intensity (∗) suggests an equilibrium between surface-bound and a more mobile phase.

Figure 2D shows the proton-decoupled ^{15}N solid-state NMR spectrum of the hydrophobic N-terminal domain of Vpu$^{1-27}$, a peptide which is important during the life cycle of HIV-1. The isolated domain has previously been shown to form channels in biological membranes and to adopt a transmembrane helical structure.[26] In aligned membranes the labeled site exhibits ^{15}N chemical shifts at 210 ppm[26] and the $\sigma_\perp$ value of the powder pattern at 78 ppm agrees well with its transmembrane orientation (Figure 2F).

Here we present an analysis of topological and dynamic information from non-oriented membrane polypeptide samples. The approach provides topological information from proteins or peptides that exhibit fast rotational diffusion around the membrane normal also in cases were the polypeptides are difficult to reconstitute into oriented lipid bilayers. As the NMR spectral line shape is a function of the motional averaging the method also tests the dynamic properties and provides information on peptide aggregation.[11] As powder pattern samples offer little resolution the method works best when polypeptides labeled at a single site or within a uniform domain are investigated. On the other hand the accessibility of topological information from non-oriented samples has distinct advantages as, in contrast to aligned membranes, the preparation of vesicles or lipid pellets is easy and fast and allows for full or reduced hydration as well as a wide range of buffer compositions (salt, pH).

Although in non-oriented powder patterns the signal intensity is spread over a larger chemical shift range this is compensated by the improved filling factor of the coil. In contrast to aligned samples neither glass plates nor plastic sheets are needed inside the active volume nor is it critical to add about 70 wt % water as in bicellar preparations. Not being limited by the availability of sample we have recorded the spectra with our conventional NMR probes of 7 mm diameter, but smaller volumes could also be used which allows for concomitantly smaller and thereby more sensitive NMR probe geometries.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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