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Communication

Rotational diffusion of membrane proteins in aligned phospholipid bilayers by solid-state NMR spectroscopy

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Abstract

Solid-state NMR experiments on mechanically aligned bilayer and magnetically aligned bicelle samples demonstrate that membrane proteins undergo rapid rotational diffusion about the normal in phospholipid bilayers. Narrow single-line resonances are observed from ^{15}N labeled sites in the trans-membrane helix of the channel-forming domain of the protein Vpu from HIV-1 in phospholipid bilayers with their normals at angles of 0°, 20°, 40°, and 90°, and bicelles with their normals at angles of 0° and 90° with respect to the direction of the applied magnetic field. This could only occur if the entire polypeptide undergoes rotational diffusion about the bilayer normal. Comparisons between experimental and simulated spectra are consistent with a rotational diffusion coefficient (D_R) of approximately $10^5\ s^{-1}$.

Keywords: Solid-state NMR; Aligned sample; Phospholipid bilayer; Membrane protein; Rotational diffusion

The highly asymmetric environment of phospholipid bilayers suggests that rotational diffusion is an important parameter for membrane proteins. Optical and electron paramagnetic spectroscopic measurements indicate that polytopic membrane proteins undergo global rotational diffusion about the bilayer normal with coefficients (D_R) between 10^3 and $10^4\ s^{-1}$ in reconstituted phospholipid vesicles and cell membranes [1]. In this communication, we utilize solid-state NMR spectra from ^{15}N labeled backbone sites in a membrane protein to characterize its global rotational diffusion in mechanically aligned bilayers and magnetically aligned bicelles.

We have determined the three-dimensional structure of the channel-forming domain of the protein Vpu from HIV-1 in mechanically aligned bilayers [2] and in magnetically aligned bicelles (unpublished results). No molecular motion is needed to obtain high resolution NMR spectra from bilayer samples with their normals aligned parallel to the field; however, rapid rotational diffusion about the axis of alignment is essential for samples aligned perpendicular to the field, such as ''unflipped'' bicelles [3]. Previous results obtained on samples of synthetic helical peptides in mechanically aligned bilayers with their normals parallel and perpendicular to the field [4–6] suggest that trans-membrane peptides undergo rotational diffusion about the bilayer normal. However, in the absence of a three-dimensional structure there is ambiguity as to whether the rotational motion is about the bilayer normal or the long axis of the helix. Moreover, such spectra can not be used to measure the rotational diffusion coefficient; the 0° orientation gives single-line resonances in the absence of motion, and the 90° orientation is insensitive to the rate of rotational diffusion when the N–H bond vectors align

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parallel to the bilayer normal, which is a common situation for trans-membrane helices with small tilt angles.

The ^{15}N NMR spectra of the channel-forming domain of Vpu in mechanically aligned phospholipid bilayers between glass plates tilted along with the "flat" RF coil at 0°, 20°, 40°, and 90° orientations shown in Fig. 1 demonstrate that the polypeptide undergoes rapid rotational diffusion about the bilayer normal. The resonances are single lines and their frequencies change with sample alignment. The spectrum obtained at the parallel (0°) orientation has its resonance at 215 ppm, which is consistent with Leu11, the labeled residue, having a backbone N–H bond approximately parallel to the bilayer normal (and the direction of the magnetic field), as expected for a residue in a trans-membrane α-helix. The resonance line width of ~4 ppm is typical of those observed in mechanically aligned samples of membrane proteins and peptide single crystals. If the polypeptide were immobile on the timescales of the chemical shift interactions, then broad, distorted partial "powder pattern" line shapes would be observed at all sample orientations, except 0°. Thus, it is notable that single-line resonances are observed when the samples are tilted with their normals at angles of 20°, 40°, and 90°.

There are noticeable differences in the line widths of the resonances in the experimental spectra in Fig. 1. The resonances from the samples tilted at 20° and 40° are broader than those at 0° and 90°. There are several plausible explanations for this effect. The 11 mm × 11 mm × 2 mm glass plate samples are relatively large, and the 20° and 40° configurations may exhibit broadening due to uncorrected magnetic field gradients. Also, since the radiofrequency irradiations have reduced efficiency at these coil geometries, the heteronuclear decoupling may not be complete. Thus, even though the experimental setup was optimized for each sample, it is not possible to rule out experimental contributions to the line broadening using presently available instrumentation. A second potential source of differential line broadening is the mosaic spread of the sample alignment [7]. However, this is ruled out by the data in Fig. 2 where all of the resonances in samples with multiple labeled sites have the same line widths.

The most likely explanation for the variation in line widths as a function of the angle between the bilayer nor-

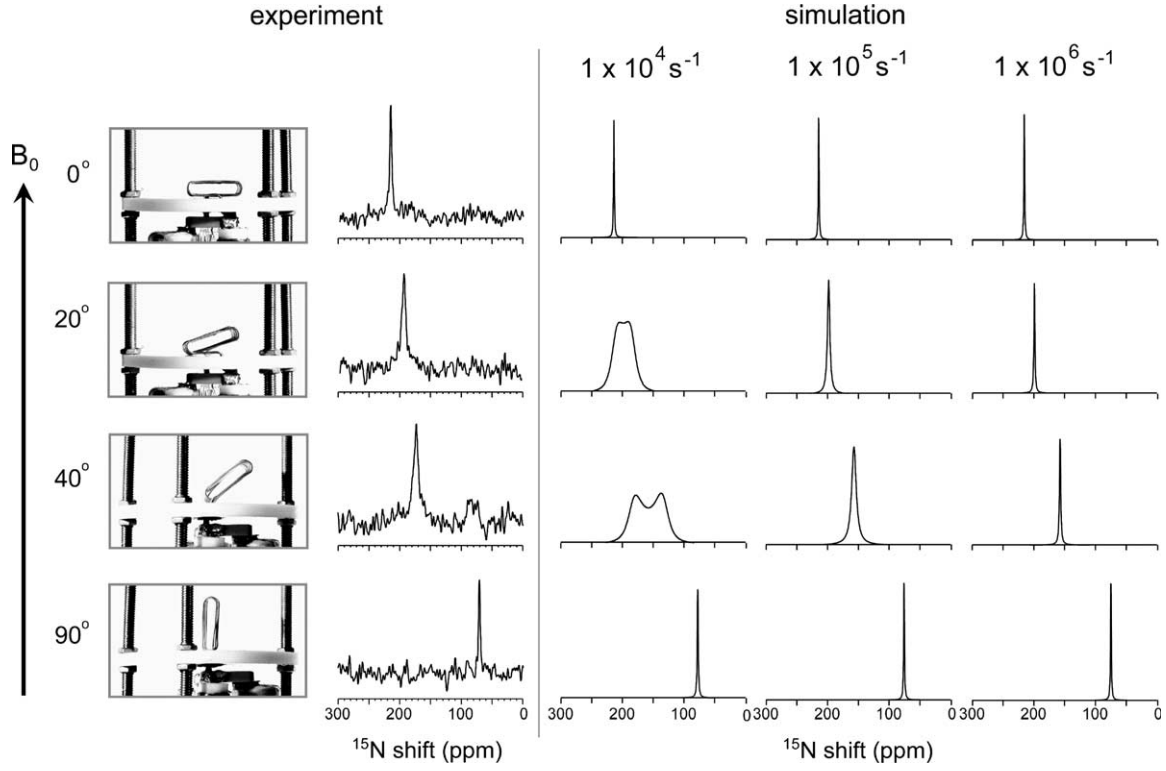

Fig. 1. Experimental and simulated ^{15}N chemical shift NMR spectra of ^{15}N Leu11 labeled trans-membrane helix of Vpu in C18:1 lipid bilayers on glass plates [2] aligned at various angles with respect to the magnetic field as shown in photographs. The trans-membrane domain of Vpu has the sequence QPIQIAIVALVVAIIIAIVVWSIVIIEGRGGKKKK, where the underlined residues are from native Vpu of BH101 isolate and six additional residues GGKKKK are added to enable the isolation, purification, and sample preparation of this hydrophobic polypeptide [2]. The 3 mg of peptide/75 mg of lipid (1:105, molar ratio) mixture was spread onto 15 glass plates, each slide measuring 11 mm × 11 mm with a 60–80 μm thickness (Marienfield, Germany) and dried completely by using a vacuum pump, and then hydrated to 95% of relative humidity in a chamber saturated with ammonium phosphate (pH 7.0) at 42 °C. The experimental spectra were obtained by cross-polarization at a ^{15}N resonance frequency of 76.015 MHz using a 0.5 ms mix time, 6 s recycle delay at 22 °C. The data were acquired for 5 ms with 50 kHz of SPINAL ^{1}H decoupling, 2–4 K transients were co-added, and Fourier transformed following apodization with an exponential function corresponding to 100 Hz of line broadening.

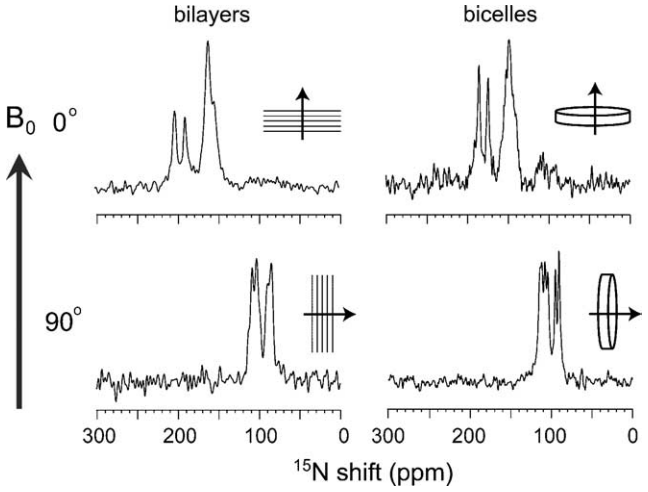

Fig. 2. ^{15}N chemical shift NMR spectra of ^{15}N Val (residues 9, 12, 13, 20, 21, and 25) labeled trans-membrane helix of Vpu in C14:0 lipid bilayers [2] and bicelles [3] at 0° and 90° sample orientations. The experimental conditions of the bilayer sample are the same as in Fig. 1. Three milligrams of labeled protein was incorporated into bicelles consisting of ether-linked phospholipids (14-O-PC/6-O-PC = 3.2, 28% w/v) at a 1:80 molar ratio. The ether linked lipids were from Avanti. Three millimolars of $YbCl_3$ (Sigma) was added to flip the bicelle sample (0° orientation). The spectra of the bicelle samples were obtained using cross-polarization with a 1 ms mix time and 7 s recycle delay at 40 °C. Data were acquired for 10 ms with 54 kHz of SPINAL ^{1}H decoupling, and 1 K transients were co-added.

mal and the magnetic field is illustrated by the comparisons of experimental and simulated spectra in Fig. 1. A formalism developed for the uniaxial motional averaging of chemical shift anisotropy [8] was applied to a resonance in a uniaxially aligned protein. The simulated spectra show that when the bilayer normal is parallel to the magnetic field (0° orientation), rotational diffusion has no impact. Rotational diffusion is essential to obtain single-line resonances when the bilayer normal is tilted at any other angle. In the absence of rotational diffusion about the bilayer normal, a cylindrical powder pattern results for the 90° orientation [3]; the simulations in Fig. 1 indicate that $D_R > 10^4\ s^{-1}$ results in complete averaging. At frequencies near the span of the ^{15}N amide chemical shift tensor ($10^4 s^{-1}$–$10^5\ s^{-1}$), the shapes and widths of the resonances for the 20° and 40° orientations reflect incomplete motional averaging of the underlying powder pattern.

^{15}N NMR spectra of samples with multiple labeled sites in mechanically aligned bilayers and magnetically aligned bicelles at 0° and 90° orientations are compared in Fig. 2. The complete line narrowing for all resonances at the 90° orientation confirms that the proteins undergo rotational diffusion faster than $10^4\ s^{-1}$ [3,8]. The resonances from proteins in magnetically aligned bicelles have narrower line widths than those in mechanically aligned bilayers. Some of this is due to the 0.8 order parameter, which reflects the "wobble" of bicelles [3]. In Fig. 2, it is the uniformity of the line widths of the resonances in the individual spectra that demonstrate that broadening observed at the 20° and 40° orientations in Fig. 1 is not due to angular-dependent effects of mosaic spread, since a range of N–H bond vector orientations are present in the multiply labeled samples.

The simulated spectra with $D_R = 10^5\ s^{-1}$ are most similar to the experimental data. Since we cannot completely rule out line broadening due to experimental factors, this places a lower limit on the rotational diffusion coefficient of the channel-forming domain of Vpu. As evidenced by the data in Fig. 2, this rate of rotation is more than adequate for obtaining high resolution NMR spectra from "unflipped" bicelles at the 90° orientation. We have observed experimental line widths of ~1 ppm for polytopic membrane proteins at both 0° and 90° orientations of bicelles. The resulting high resolution spectra and experimental flexibility contribute to the utility of solid-state NMR of aligned samples as a method for determining the atomic-resolution structures of membrane proteins in fully hydrated planar phospholipid lipid bilayers under physiological conditions.

Acknowledgments

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