New and Notable

New Angles for NMR Studies of Biological Molecules

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The biological functions of membrane-associated molecules are closely related to their conformations. The use of variable angle sample spinning (VASS) in the NMR studies of these molecules facilitates the extraction of structural information.

The determination of the structure of a biological molecule is an essential step in understanding how it functions. Nuclear magnetic resonance is one of the most useful techniques for obtaining information on molecular structures. For NMR studies of water-soluble molecules, bond distances and bond angles are usually determined by studying the nuclear Overhauser effect in aqueous solutions or residual dipolar coupling constants in weakly ordered aqueous media. On the other hand, many important proteins are embedded in cell membranes, rather than being freely tumbling in the aqueous phase, and require different NMR techniques for their structural determination.

The method of magic angle spinning (MAS; Fig. 1, in which $\beta = \tan^{-1}\sqrt{2} = 54°44'$) is widely used in solid-state NMR. With MAS at high enough spinning rates and ^{1}H broadband decoupling, the NMR spectra of rare spin 1/2 nuclei (mainly ^{13}C, ^{15}N, and ^{31}P) show narrow peaks characterized by chemical shifts only. For the purpose of structural determination, dipolar couplings can be recovered by using rotor-synchronized pulses (REDOR); chemical shift anisotropy information can be obtained from spinning sidebands, but it requires the use of lower spinning rates, which may result in broader peaks.

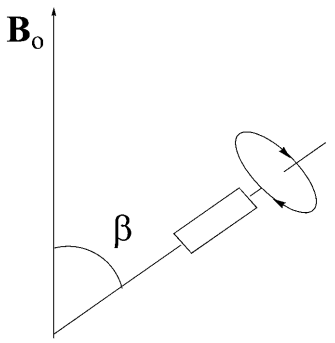

FIGURE 1 A schematic diagram of variable sample spinning.

When model membrane systems are prepared in the form of lipid dispersions, techniques other than MAS are often used. When these samples are placed in a magnetic field, there may or may not be a macroscopic alignment, depending on the competition between two torques. The magnetic torque tends to make the local order director of a liquid crystal align either parallel (for samples with positive anisotropy of the magnetic susceptibility, namely $\Delta\chi > 0$) or perpendicular (for $\Delta\chi < 0$) to the magnetic field. The viscous torque tends to counteract this alignment. If the lipid dispersion is in the lamellar phase, the viscous torque prevails, and the sample does not adopt macroscopic ordering in a magnetic field; however, static samples with uniform orientation can be prepared by using thin glass slides for alignment. If the dispersion is in the nematic phase or a perforated lamellar phase such as bicelles (Gaemers and Bax, 2001), macroscopic alignment in a high magnetic field is usually observed. In either case, chemical shift anisotropy and dipolar coupling constants can be determined from the NMR spectra.

For liquid crystals that automatically align in the magnetic field, the angle of alignment of the director can be controlled by using variable angle sample spinning (Fig. 1). In this case, the balance of the two torques would cause the director to orient so that the average potential energy is at the minimum (Courtieu et al., 1982). If the spinning rate is sufficiently high (>300 Hz at 11.7 T for most nematic and bicellar systems, but 1–2 kHz is preferable), the orientation of the director obeys the relations with respect to the spinning axis as shown in Table 1.

For parallel alignment, the director of the liquid crystal has a uniform orientation with respect to the magnetic field, and the NMR peaks remain quite sharp; in the meantime, all anisotropic interactions (chemical shift, dipolar coupling, quadrupolar coupling) are reduced by a factor of $(3\cos^2\beta - 1)/2$. For perpendicular alignment, the reduction factor is $-(3\cos^2\beta - 1)/4$; however, the director has a mosaic spread rather than a uniform orientation with respect to the magnetic field, and the NMR peaks exhibit a partial powder pattern, somewhat similar to the situation of off magic angle spinning for solids. At the magic angle, the director has no preferred orientation, but the NMR peaks are still quite sharp because the factor $(3\cos^2\beta - 1)$, which affects all anisotropic interactions, equals zero.

Taking advantage of the reduction of anisotropic interactions, the VASS technique has been applied rather extensively to determine ^{1}H-^{13}C dipolar coupling constants in thermotropic liquid crystals (Courtieu et al., 1994). More recently, the potential of using VASS to study lyotropic bicellar liquid crystals as model systems for membrane-associated biomolecules has been explored (Zandomeneghi et al., 2001, 2003). In this issue of the *Biophysical Journal*, Kishore and Prestegard (2003) report the use of VASS NMR to obtain information on ^{31}P chemical

TABLE 1

	$\Delta\chi < 0$	$\Delta\chi > 0$
$\beta < 54°44'$	Perpendicular	Parallel
$\beta > 54°44'$	Parallel	Perpendicular

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shift anisotropy of two phosphatidylinositol phosphates embedded in a lyotropic liquid crystalline membrane mimic.

Phosphatidylinositol biphosphate ($PI(4,5)P_2$) is a phosphatidylinositide functioning in the regulation of many mammalian cellular processes. It contains three phosphate groups, one of them with a diester linkage and two being monoester head groups. With VASS of the membrane-like bilayer system, the three corresponding ^{31}P NMR signals are well resolved. Plots of the observed ^{31}P chemical shifts versus $(3\cos^2\beta - 1)$ yield two linear segments intersecting at the magic angle, as expected. The slopes give the values of the anisotropic contribution to the chemical shift, $\Delta\delta_{ansio} = \delta_{oriented} - \delta_{iso}$. Phosphatidylinositol-4-phosphate (PI(4)P) has two phosphate groups, one being a diester linkage and the other a monoester head group. The $\Delta\delta_{ansio}$ values for the two ^{31}P peaks are obtained similarly.

Unlike dipolar coupling constants, chemical shifts anisotropy values cannot be used directly to obtain structural information. However, constraints of molecular geometry can be inferred from the data by comparison with the results of molecular modeling. Starting with a set of reasonable chemical shift tensors and molecular conformations, Kishore and Prestegard carried out computations for a small set of rotatable torsion angles using a molecular modeling program called Sybyl (Sybyl 6.7, Tripos, St. Louis, MO). By comparing the calculated values with the experimental data, they were able to suggest a few possible conformations for each of the two phosphatidylinositides in the lipid bilayer.

Although the study does not give a definitive geometry for either molecule, the authors point out that they have demonstrated the usefulness of applying the VASS technique as a new tool for the NMR study of membrane-associated biological molecules. They also suggest that the measurement of ^{13}C and ^{15}N chemical shift anisotropies would provide more valuable information. It should also be pointed out that the use of VASS to study dipolar couplings would yield structural information more directly and complement other NMR techniques well.

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