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A liquid crystalline medium for measuring residual dipolar couplings over a wide range of temperatures

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Abstract

A mixture of dilauroyl phosphatidylcholine (DLPC) and 3-(cholamidopropyl)dimethylammonio-2-hydroxyl-1-propane sulfonate (CHAPSO) in water forms disc shaped bicelles that become ordered at high magnetic fields over a wide range of temperatures. As illustrated for the FK506 binding protein (FKBP), large residual dipolar couplings can be measured for proteins dissolved in low concentrations (5% w/v) of a DLPC/CHAPSO medium at a molar ratio of 4.2:1. This system is especially useful for measuring residual dipolar couplings for molecules that are only stable at low temperatures.

Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DHPC, dihexanoyl phosphatidylcholine; DLPC, dilauroyl phosphatidylcholine; CHAPSO, 3-(cholamidopropyl)dimethylammonio-2-hydroxyl-1-propane sulfonate; FKBP, FK506 binding protein.

The three-dimensional structures of macromolecules that are determined by NMR spectroscopy are largely constrained by short distances derived from nuclear Overhauser effects. Unfortunately, the use of these short distance constraints limits the accuracy of NMR-derived structures, especially for non-globular molecules where the cumulative error can be significant. Recently, angular restraints obtained from residual dipolar couplings (Kung et al., 1995; Tolman et al., 1995; Tjandra et al., 1996, 1997; Tolman and Prestegard, 1996; Tjandra and Bax, 1997a) have been used in NMR-based structure calculations (Tjandra and Bax, 1997b). Unlike NOEs, the angles derived from residual dipolar couplings are all relative to a common axis and are distance-independent. Thus, these restraints may improve the accuracy of NMR-derived structures (Tjandra and Bax, 1997b).

The magnitude of residual dipolar couplings depends on the ordering of a molecule in a strong magnetic field. Most diamagnetic molecules align only weakly in the magnetic field, resulting in small residual dipolar couplings (< 0.5 Hz) that are difficult to accurately measure. Recently, Tjandra and Bax demonstrated a valuable approach for increasing the size of residual dipolar couplings by partially ordering a protein in a dilute liquid crystalline medium (Tjandra and Bax, 1997b; Bax and Tjandra, 1997). In these studies, disc-like bicelles are formed from a mixture of dimyristoyl phosphatidylcholine (DMPC) and dihexanoyl phosphatidylcholine (DHPC) (Sanders and Schwonek, 1992). The bicelles orient spontaneously in a strong magnetic field and cause the partial alignment of proteins in the solution. This results in an increase in the observed residual dipolar couplings. Although useful for many proteins, a potential drawback of the DMPC/DHPC system is the requirement of a relatively high temperature ($> 30\,^{\circ}$C) for alignment of the bicelles in the magnetic field. This may preclude the use of this system for studying molecules that are unstable at high temperatures.

In this communication, we describe the use of a stable liquid crystalline system for measuring resid-

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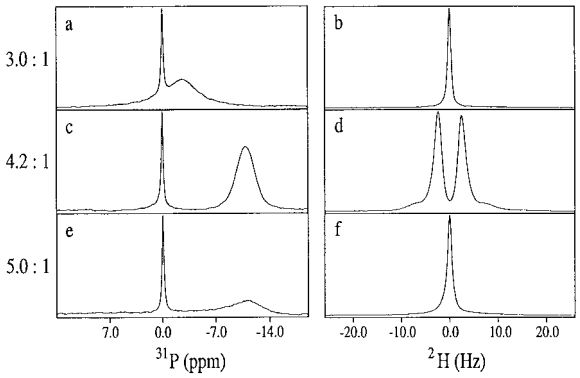

Figure 1. ^{1}H decoupled (1.25 kHz decoupling power) ^{31}P NMR spectra (a,c,e) and ^{2}H NMR spectra (b,d,f) of the DLPC and CHAPSO mixture acquired at 27 °C with different DLPC/CHAPSO molar ratios. The samples contain 5% (w/v) total DLPC and CHAPSO in a 50 mM phosphate buffered (pH 6.8) solution containing 50 mM KCl and 10% D_2O. The spectra were recorded on a Bruker DRX600 NMR spectrometer. The ^{31}P NMR spectra are referenced relative to the ^{31}P resonance of the phosphate buffer. Both the DLPC and CHAPSO were purchased from Sigma in powder form. The lipid samples were prepared by dissolving DLPC and CHAPSO in the phosphate buffer and mixing vigorously by vortexing the mixture at high speed for at least 1 min. The samples were transferred to 5 mm NMR tubes and were allowed to stabilize (> 1 h) before recording the NMR spectra. The sample with a 4.2:1 DLPC/CHAPSO ratio was stable at room temperature for a few weeks. However, the sample with a 3:1 DLPC/CHAPSO ratio separated into two phases after about 3 h.

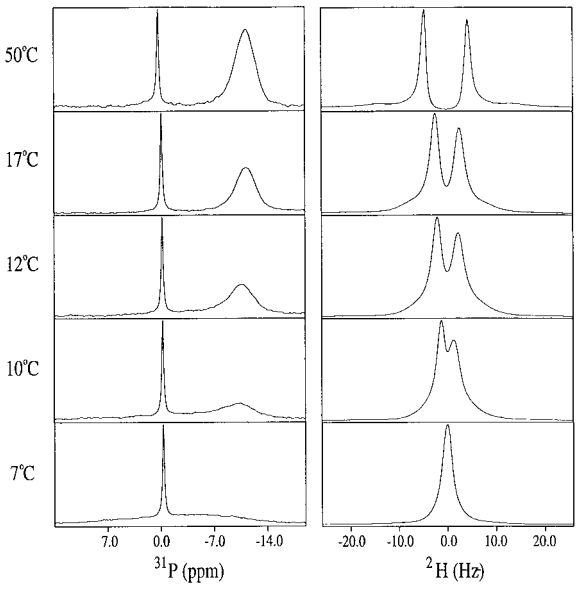

Figure 2. ^{31}P (^{1}H decoupled, 1.25 kHz decoupling power) and ^{2}H NMR spectra of the DLPC and CHAPSO mixture at a ratio of 4.2:1 acquired at different temperatures on a Bruker DRX600 NMR spectrometer.

ual dipolar couplings at lower temperatures. It is based on the liquid crystal formed by a mixture of dilauroyl phosphatidylcholine (DLPC) and the zwitterion 3-(cholamidopropyl)dimethylammonio-2-hydroxyl-1-propane sulfonate (CHAPSO). In addition to characterizing the proper experimental conditions for employing this liquid crystalline medium, we demonstrate its utility by measuring residual ^{1}H–^{15}N dipolar couplings for the FK506 binding protein (FKBP).

Like the DMPC/DHPC system, DLPC and CHAPSO can form bicelles that orient spontaneously in a strong magnetic field. Indeed, DLPC/CHAPSO mixtures have been used in solid-state NMR to study membrane-associated proteins (Sanders et al., 1994). For solid-state NMR, high concentrations (30% w/v) of DLPC/CHAPSO at a molar ratio of 3:1 was used. For high-resolution NMR studies, however, it is necessary to decrease the concentration of the lipid to obtain narrow linewidths and a simple spectrum (Bax and Tjandra, 1997). As with other lipid mixtures, the ability of DLPC and CHAPSO to form an ordered state in a strong magnetic field depends on the molar ratio of the molecules. As shown in Figure 1, a DLPC/CHAPSO mixture at a molar ratio of 3:1 (the ratio used in the solid state NMR experiments) and a total lipid concentration of 5% (w/v) is in an isotropic phase and does not order in the magnetic field. Both the ^{31}P NMR spectrum of DLPC (Figure 1a) and the ^{2}H NMR spectrum of the HDO solvent (Figure 1b) show broad symmetric singlets, such as those typically observed in an isotropic medium. In contrast, at a DLPC/CHAPSO molar ratio of 4.2:1, the lipids form bicelles that partially orient in the magnetic field. A -12 ppm upfield shift of the ^{31}P signal (Figure 1c) indicates that the bicelles are oriented with their normals orthogonal to the magnetic field (Seelig et al., 1985). The deuterium spectrum of HDO in the sample (Figure 1d) appears as a doublet, reflecting the incomplete averaging of the large ^{2}H quadrupolar coupling caused by the anisotropic medium. However, when the DLPC/CHAPSO molar ratio was raised further to 5:1, the mixture became cloudy and did not order in the magnetic field. Under these conditions, the ^{31}P NMR spectrum (Figure 1e) became very broad, and the ^{2}H NMR spectrum of HDO (Figure 1f) consists of a singlet.

Figure 2 shows the temperature dependence of the ^{31}P and ^{2}H NMR spectra of the DLPC/CHAPSO liquid crystal (4.2:1 molar ratio, 5% total w/v concentration). At 7 °C or below, the lipid system is in

the isotropic phase. Above 7 °C, the lipids are ordered in the magnetic field. The splitting of the deuterium signal becomes larger as the temperature is increased. The transition between the isotropic phase and ordered phase is reversible (data not shown), and the DLPC/CHAPSO lipid crystalline medium is stable for weeks at room temperature.

The utility of the DLPC/CHAPSO liquid crystalline medium for measuring residual dipolar couplings is illustrated for the FK506 binding protein (MW = 12 kDa). Figure 3 depicts a small region of a ^{1}H–^{15}N HSQC spectrum of uniformly ^{15}N-labeled FKBP acquired in the presence of the lipid mixture. The spectrum was recorded on a Bruker DRX800 NMR spectrometer without ^{1}H decoupling during the ^{15}N evolution period. Thus, the size of the observed splitting in the ^{15}N dimension corresponds to the sum of the scalar coupling ($^{1}J_{\mathrm{NH}}$) and the residual dipolar coupling ($^{1}D_{\mathrm{NH}}$) constants. Since $^{1}D_{\mathrm{NH}}$ is negligible for FKBP in the absence of lipid (< 0.5 Hz, data not shown), the difference of the splittings relative to FKBP without lipids corresponds to residual dipolar coupling constants in the presence of the liquid crystal system. As shown in Figure 4, the measured values range from -11 Hz to 10 Hz and fit well (correlation coefficient = 0.92) to the values calculated from the crystal structure of FKBP (PDB access code 1 FKK) based on the equation (Tjandra and Bax, 1997b):

$$^{1}D_{\mathrm{NH}} = -S\frac{\mu_0}{4\pi}\frac{\gamma_N\gamma_H h}{4\pi^2 r^3} \times \left[A_{\mathrm{a}}(3\cos^2\theta - 1) + \frac{3}{2}A_{\mathrm{r}}(\sin^2\theta \times \cos 2\phi)\right] \quad (1)$$

where S is the generalized order parameter for internal motion of the N–H vector, γ_{N} and γ_{H} are the magnetogyric ratios of ^{15}N and ^{1}H, h is Planck's constant, r is the N–H bond length, A_{a} and A_{r}, are the axial and rhombic components of the molecular alignment tensor, and θ and ϕ are cylindrical coordinates of the N–H bond vector in the principal axis system of A.

In summary, we have shown that a DLPC/CHAPSO mixture at a low concentration (5%) forms bicelles that can cause proteins to partially orient in a strong magnetic field. As illustrated for FKBP, this results in a dramatic increase in the magnitude of the residual dipolar couplings and allows these couplings to be easily measured. Unlike the DMPC/DLPC liquid crystal system, DLPC/CHAPSO forms an ordered state over a wide range of temperatures and is especially useful

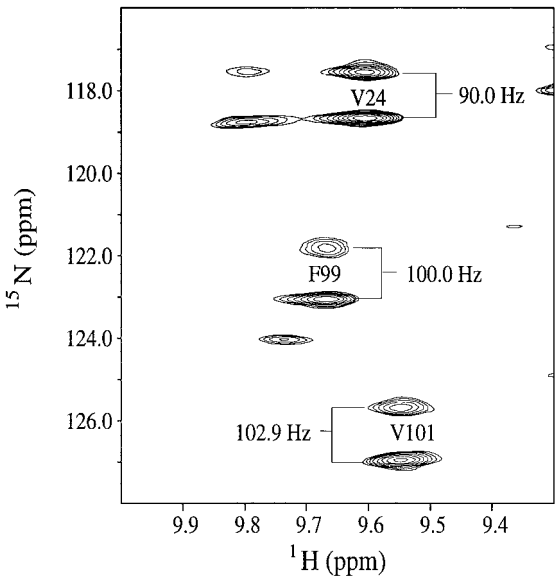

Figure 3. Small region of a ^{1}H–^{15}N HSQC spectrum of FKBP acquired in the presence of DLPC/CHAPSO without ^{1}H decoupling in the ^{15}N dimension. The size of $^{1}J_{\mathrm{NH}} + ^{1}D_{\mathrm{NH}}$ splittings (Hz) is indicated. The spectrum was recorded at 17 °C on a Bruker DRX800 NMR spectrometer. The sample was prepared by diluting a 10% (w/v) DLPC/CHAPSO mixture (prepared as described in the legend of Figure 1) by the addition of an equal volume of a 1 mM solution of FKBP dissolved in the same buffer. The solution was then mixed at room temperature by gentle vortexing. The final sample contained 0.5 mM FKBP and 5% w/v total DLPC/CHAPSO at a molar ratio of 4.2:1.

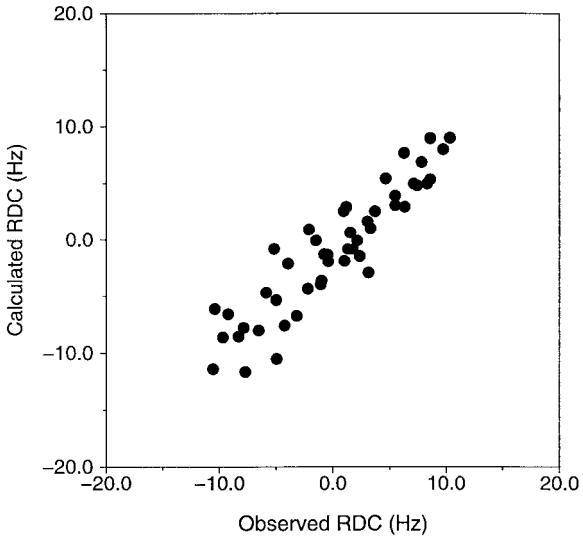

Figure 4. The correlation of the measured residual ^{1}H–^{15}N dipolar couplings of the uniformly ^{15}N labeled FKBP in the DLPC/CHAPSO liquid crystalline medium (as described in Figure 3) to the values fitted to the crystal structure of FKBP. The correlation coefficient is 0.92.

for measuring residual dipolar couplings in molecules that are only stable at low temperature.

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