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Journal of Magnetic Resonance 159 (2002) 82–86
JMR Journal of Magnetic Resonance
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Communication

Capture and manipulation of magnetically aligned Pf1 with an aqueous polymer gel

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Received 28 January 2002; revised 5 August 2002

Abstract

The magnetic alignment of the Pseudomonas bacteriophage Pf1 is captured indefinitely in a gel of the aqueous triblock copolymer Pluronic F-127. In addition to preserving high-resolution liquids NMR spectra for dissolved solutes, the gel prevents the reorientation of the phage allowing mechanical manipulation of the angle between the axis of the phage alignment and the static magnetic field. The residual ^{2}H quadrupolar couplings for several solutes dissolved in this material as a function of the angle Θ between the non-spinning sample tube and the static magnetic field are consistent with the value of $P_2(\cos\Theta) = (3\cos^2\Theta - 1)/2$. The variable–angle correlation spectrum for these solutes is shown to separate residual quadrupolar effects from isotropic chemical shifts. Finally, the compatibility of Pluronic F-127 with NMR studies of aqueous biological macromolecules is demonstrated in a measurement of residual dipolar couplings in an ^{15}N-labeled nucleic acid.

Keywords: Alignment; Residual couplings; Variable–angle NMR; Bacteriophage; RNA; Pluronic F-127

1. Introduction

The refinement of macromolecular structure has been revolutionized by the measurement of residual dipolar couplings and their subsequent use as bond vector constraints in chemical structure calculations. Several methods routinely used to recover these residual effects in the liquids nuclear magnetic resonance (NMR) spectra of biological macromolecules while maintaining high resolution involve using liquid crystalline solvents such as magnetically oriented phospholipid bicelles [1], bacteriorhodopsin purple membranes [2], rod-shaped viruses [3], or bacteriophage [4]. While each of these methods partially restore full anisotropy to the liquids NMR spectrum of co-solutes dissolved in the liquid crystal (LC), the inherent orientational degeneracy of solute alignment tensors along the LC director leads to ambiguity in the direct application of residual couplings to chemical structure refinement [5,6]. To alleviate this problem, different alignment media with independent LC directors have been used [5,6], although this approach is obviously not applicable in most situations where only limited quantities of sensitive biological samples are available. Both chemical and mechanical methods of sample manipulation have been proposed to modify the LC director in just one sample [2,5–8]. The chemical approach involves varying the ionic strength of the solution [2,7], changing the net charge of the magnetically anisotropic solute [5,6], or doping with paramagnetic lanthanide ions [8]—strategies that may destroy or change the macromolecular structure of the compound under investigation. The mechanical approach endeavors to switch the director axis with respect to the magnetic field by physically moving the sample and has relied on using either organic LC's with long-orientational relaxation times [9,10] or alignment in the shear field inside a spinning rotor inclined away from the direction of B_0 [11,12]. Unfortunately, the timescale of several seconds required for these experiments prohibits direct extension to the use of biologically compatible LCs that orient on faster timescales.

An alternative approach that is applicable to sensitive biological samples and is an extension of earlier work for

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PII: S1090-7807(02)00004-6

purple membranes captured in polyacrylamide gels [13] and for solutes in strained polyacrylamide gels [14,15] is presented here. This study considers the variable–angle director manipulation of solutes in a suspension of the Pseudomonas bacteriophage Pf1 constrained in a polymer gel. To date, Pf1 solutions have become the most widely used aqueous-phase LC solvents for macromolecular alignment. Furthermore, it is known that the Pf1 molecules maintain alignment in polyacrylamide gels [16,17]. Given the toxicity of polyacrylamide and the irreversibility of polyacrylamide crosslinking, a more suitable polymer matrix is formed here with the non-toxic commercially available aqueous surfactant Pluronic F-127 [18]. At weight-to-volume (w/v) concentrations greater than 20% in water, this triblock copolymer consisting of poly(propylene oxide) and poly(ethylene oxide) chains is a flowing liquid at 4 °C and a viscous gel at room temperature [19]. The viscosity transition is reversible making it easy to induce hardening of the solution within the magnetic field by warming and to recover macromolecular solutes through cooling and centrifugation.

The effect of Pluronic F-127 gel on the residual quadrupolar splitting of D_2O in the one-dimensional (1D) ^2H spectra of a Pf1 solution containing small fractions of D_2O, CD_3OD, and CD_3CN in a 5 mm diameter NMR tube with its long axis parallel to B_0 is shown in Fig. 1. The splitting observed in the HOD spectral region in the absence of the gel in A is consistent with that in a 20% w/v gel in B. While a slight increase in line width is observed, the high resolution of liquids NMR is retained. A residual quadrupolar splitting is also observed for CD_3CN but not for CD_3OD suggesting that the CD_3OD is isolated from the aligned phage by the Pluronic F-127. Fig. 2A demonstrates the effect of rotating the long axis of the sample tube away from the direction of B_0 by the angle Θ on the two residual quadrupolar splittings in this sample. It is clear that both the D_2O and CD_3CN residual splittings shown as circles and diamonds, respectively, modulate with the angle Θ, and as shown by the solid lines in Fig. 2A, excellent agreement is found between the experimental angular dependence and the value of the second-order Legendre polynomial $P_2(\cos\Theta) = (3\cos^2\Theta - 1)/2$. For comparison, the squares in Fig. 2A show the corresponding angular data for a solution containing the same Pf1 concentration in D_2O/H_2O without Pluronic F-127. This variable–angle data can also be analyzed in a two-dimensional fashion as shown in the pioneering work by Frydman, et al. [20]. The variable–angle

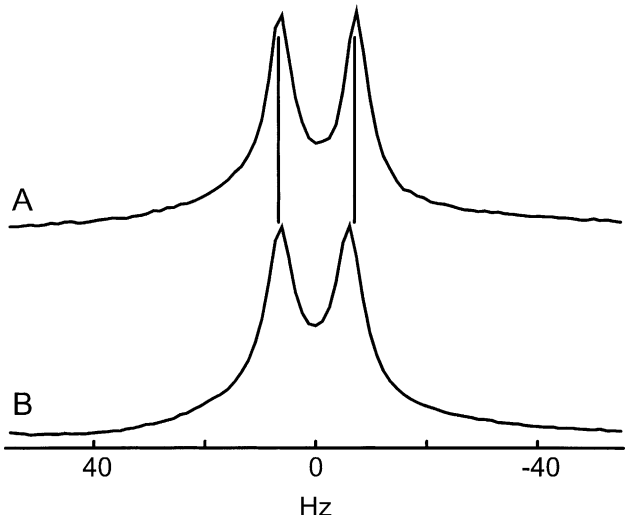

Fig. 1. ^2H NMR spectra of the HOD region showing the effects of Pluronic F-127 on the residual quadrupolar splitting of D_2O in a $\approx$ 13 mg/mL Pf1 bacteriophage solution containing fractions of D_2O, CD_3OD, and CD_3CN in H_2O. The spectrum in (B) observed in a 20% w/v Pluronic F-127 gel compares well with the spectrum obtained in the absence of the gel in (A).

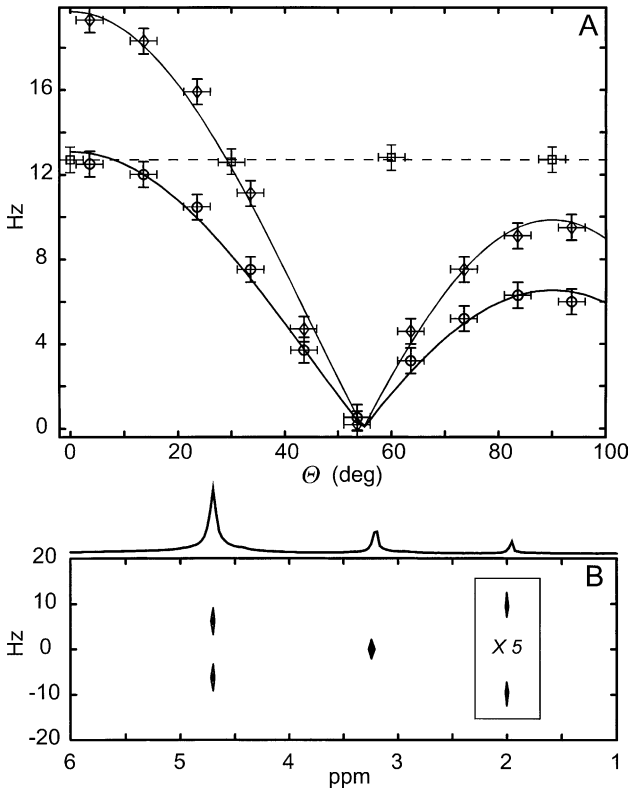

Fig. 2. Variable–angle results of gel-constrained magnetic alignment. Manual rotation of the 13 mg/mL Pf1 in a 20% w/v Pluronic F-127 sample away from the magnetic field axis by the angle Θ results in a modulation of the residual couplings at $P_2(\cos\Theta)$ as shown in (A) for D_2O (◯) and CD_3CN (◇). The solid lines correspond to the value of $P_2(\cos\Theta)$ scaled by 13 and 20 Hz for D_2O and CD_3CN, respectively. In the absence of the gel, reorientation of an unconstrained 13 mg/mL Pf1 solution results in a Θ-independent ^2H splitting as shown by the squares in (A). The 2D variable–angle correlation of isotropic chemical shifts with residual quadrupolar couplings shown in (B) can be constructed from the spectra used to generated the data shown in (A). The projection of the 2D spectrum along the isotropic axis yields the 1D isotropic gel-free spectrum where the chemical shifts for D_2O, the CD_3OD methyl deuterons, and CD_3CN are 4.7, 3.3, and 1.9 ppm, respectively.

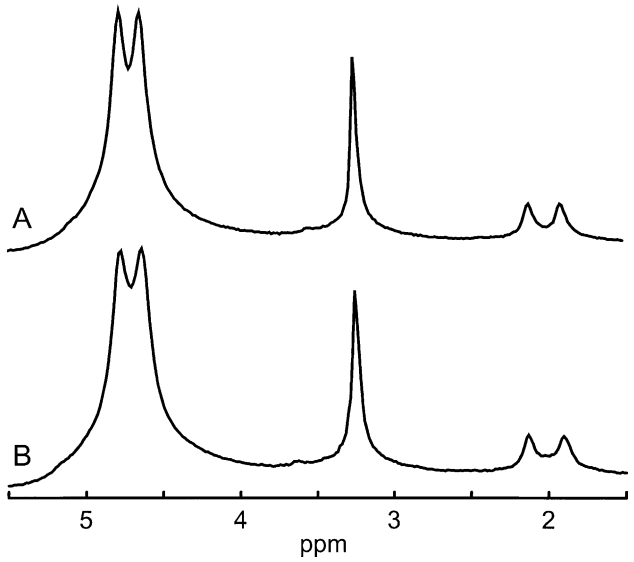

Fig. 3. The orientation of the magnetically aligned Pf1 can be captured indefinitely as shown by the comparison of a freshly cast gel in (A) with a one-week-old sample in (B). Both spectra were recorded with $\Theta = 90°$ to emphasize the trapped alignment.

correlation spectrum of the data in Fig. 2A that correlates isotropic chemical shifts with residual quadrupolar couplings is shown in Fig. 2B. The spectra shown in Fig. 3 with $\Theta = 90°$ demonstrate that constrained alignment persists in the absence of the magnetic field for at least one week. Similar results on other samples indicate that the alignment can be captured for more than 40 days. The splittings and line widths in these samples are identical to the freshly prepared sample, however, the increase in the saddle point intensity between the split lines in Fig. 3B in comparison to Fig. 3A is due to the formation of an isotropic peak. Presumably, long-time contraction of the gel from storage under varying laboratory conditions occludes the smaller solute molecules from the Pf1 while preserving the alignment of the phage particles.

The suitability of Pluronic F-127 for high-resolution NMR studies of biologically relevant molecules is suggested by diffusion studies of small oligonucleotides and DNA fragments [21,22] and is demonstrated here using a uniformly ^{15}N-labeled stem loop of the Varkud satellite (VS) ribozyme substrate RNA (5′-GGUGCGAA GGGCGUCGUCGCCCCGAGCGCC-3′) [23]. In an isotropic solution, the imino region of the 500 MHz ^{1}H spectrum shown in Fig. 4A displays ^{15}N–^{1}H scalar J couplings of 90 and 88 Hz for the respective U617 and G642 nucleotide resonances centered at 11.7 and 11.0 ppm. In the presence of 17 mg/mL Pf1, a change in the imino proton splittings in Fig. 4B to 100 and 101 Hz for the same two nucleotides indicates the recovery of residual dipolar couplings; the increase in line widths is typical of bacteriophage solutions [4]. Aligning the RNA at 2 °C in a 21 mg/mL bacteriophage solution containing

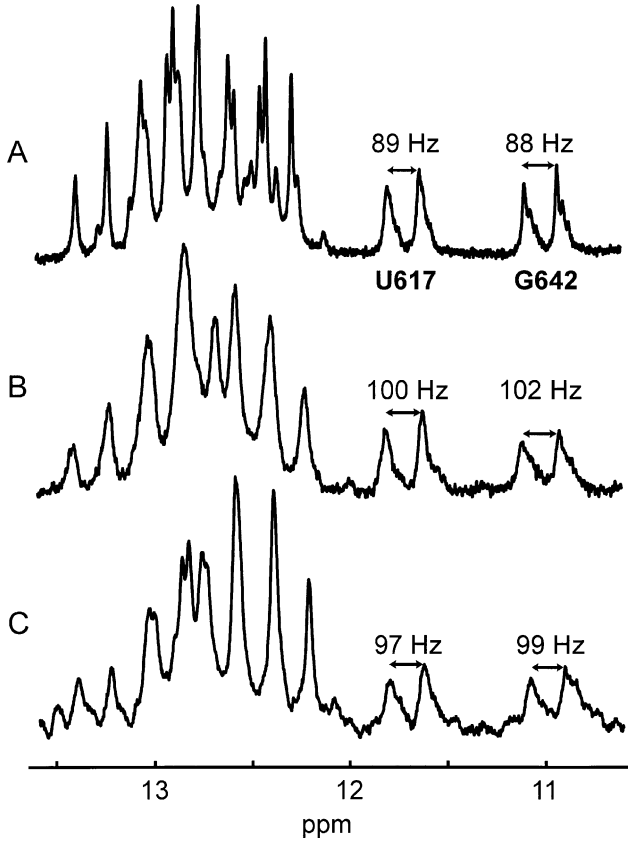

Fig. 4. Imino proton region of the 1D ^{1}H spectrum of an ^{15}N-labelled Varkud satellite stem-loop RNA showing the U617 and G642 resonances centered at 11.7 and 11.0 ppm, respectively, without phage in (A) with 17 mg/mL Pf1 in (B) and with 21 mg/mL Pf1 and 14% w/v Pluronic F-127 in (C). The benign interaction of the Pluronic F-127 polymer matrix with biological macromolecules is demonstrated by the similarity of splittings and line widths in (B) and (C). The RNA concentration (number of scans) is 1.0 (32), 0.25 (256), and 0.17 mM (2048) for A, B, and C, respectively.

14% w/v Pluronic F-127 followed by gradual warming gives the room temperature spectrum in Fig. 4C in which no loss of resolution due to the polymer matrix is observed when compared to the spectrum in Fig. 4B. Splittings of 97 and 99 Hz for the U617 and G642 imino proton resonances, respectively, still indicate the partial recovery of dipolar couplings in the presence of Pluronic F-127 gel. The slight difference in splittings in the U617 and G642 resonances as well as line shift and splitting changes in the 12–14 ppm region between all of the spectra in Fig. 4 suggest the ordering tensors describing the RNA alignment in the polymer/phage and phage systems may differ.

Given the persistent alignment and the ease of temperature-controlled gel formation of Pluronic F-127, it is interesting to speculate towards preparing and constraining magnetically aligned solutes in fields greater than 16 T followed by later NMR investigation on more common lower field spectrometers. In this way distance information obtained from residual dipolar couplings

could be measured at low field—fields where magnetic alignment is small. In addition to the usual library of 2D switched-angle NMR techniques currently being examined in this system, one can also imagine using a single sample with Pluronic F-127 to repeatedly cast LC gels with a director pointing along any axis with respect to the NMR sample container.

2. Experimental

The Pf1 bacteriophage and VS RNA were prepared using previously reported methods [4,23]. The gel samples were prepared in a cold room at 4 °C by dissolving the Pluronic F-127 into a solution containing the solute(s) of study followed by addition of the bacteriophage. For the $D_2O/CD_3OD/CD_3CN$ sample, 150 mg of powdered Pluronic F-127 surfactant was dissolved into a 225 μL solution containing equal volumes of D_2O, CD_3OD, and CD_3CN. The cold surfactant solution was then added to 500 μL of a 20 mg/mL bacteriophage solution in H_2O and mixed by repeated pipetting. The RNA/Pluronic F-127 sample was prepared by first dissolving 2.5 mg of lyophilized RNA into 300 μL of H_2O. The pH was then adjusted to 8.0 using 30 μL of 100 mM NaOH solution followed by the addition of 100 mg of Pluronic F-127. After the surfactant had completely dissolved, 300 μL of 49 mg/mL bacteriophage and 50 μL of D_2O for the spectrometer lock were added. These mixtures were then transferred to standard 5 mm liquids NMR tubes and kept cold until placed into a 6.95 T magnetic field in a Nalorac variable-temperature liquids NMR probe equilibrated to 2 °C. Alignment of the phage was tracked by monitoring the D_2O quadrupolar splitting and was found to be complete within one hour; the samples were kept at 2 °C for a total of 6 h to ensure equilibrium phage alignment. The temperature of the samples was then increased at a rate of 1 °C/min to a final temperature of 30 °C, where it was held for 12 h overnight to ensure complete gel formation. The sample tubes were removed from the magnetic field, shortened to 40 mm, and stored on the bench top.

All ^2H NMR measurements for the $D_2O/CD_3OD/CD_3CN$ sample were recorded at a resonance frequency of 45.36 MHz using a home-built NMR spectrometer controlled by a Tecmag Orion pulse programmer and built around an Oxford Instruments 6.95 T wide-bore superconducting solenoid magnet. Sixteen scans were accumulated per spectrum with a recycle delay of 1 s. The home-built variable–angle NMR probe head accommodates standard 5 mm diameter NMR tubes cut to a 40 mm length that are centered in a Delrin axle with rotation axis perpendicular to B_0. The 14 μs 90° radio frequency pulses were generated by a 16 mm diameter split-solenoid coil tuned and matched by a standard inductor–capacitor tank circuit. Proton NMR spectra of the RNA samples were acquired on a Bruker Avance-500 spectrometer equipped with an HCN triple resonance, triple-axis pulsed field gradient probe. Solvent suppression of the water peak was achieved using a 11-spin echo pulse sequence [24], while presaturation was used to reduce the intensity of the primary Pluronic F-127 resonance at 3.6 ppm. The recycle delay was also 1 s.

Acknowledgments

The authors wish to thank Paul Feldstein and Edward Re for providing the Pluronic F-127, Jeremy Flinders and Thorsten Dieckmann for assistance with the RNA, and Kurt Zilm for inspiring conversation. Support from the NSF under grant number CHE-9504655 is gratefully acknowledged. MPA is a David and Lucile Packard Foundation and Alfred P. Sloan Foundation fellow.

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