Measurement of Residual Dipolar Couplings of Macromolecules Aligned in the Nematic Phase of a Colloidal Suspension of Rod-Shaped Viruses

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Residual dipolar couplings provide long-range structural restraints for NMR structure determination of macromolecules[1] that are not accessible by most other NMR observables, which are dependent on close spatial proximity of atoms.[2] Thus, dipolar couplings provide restraints for defining the relative orientation of structural elements where the information content provided by the NOE restraints is insufficient, either due to the lack of NOEs or to the accumulation of errors over long distances.[2] In addition, the use of various different dipolar couplings (e.g., $^1D_{NH}$, $^1D_{NC'}$, $^2D_{HNC'}$, $^1D_{CH}$, $^1D_{C\alpha C'}$) along the protein backbone narrowly restricts the backbone torsion angles.[3] The measurement of residual dipolar couplings requires that the macromolecule of interest be weakly aligned in the magnetic field. Alignment can be induced in a number of ways including the magnetic field itself[4] or the use of a liquid crystalline medium.[5] In general, alignment arising from the former is very small and cannot be exploited on a routine basis.[2,4] Moderate degrees of alignment, while retaining the resolution, sensitivity, and simplicity obtained in the isotropic phase, can be obtained by dissolving macromolecules in a very dilute liquid crystalline phase[6] of lipid bicelles.[7] For biological macromolecules, the liquid crystalline medium must possess a number of key features; it has to be miscible with water, it should not bind to the macromolecule of interest, it should be stable at dilute concentrations over long periods of time, and it should only induce moderate degrees of alignment. While the bicelles have been successfully applied in a number of cases,[3,6] there are instances where the protein, possibly due to the presence of surface hydrophobic patches, adversely affects the stability of the bicelle, leading to phase separation of its components.[8] In addition, the bicelles are thermotropic, adopting a liquid crystalline phase over a narrow range of temperatures, and are only stable over a limited pH range due to acid- and base-catalyzed hydrolysis of ester bonds.[6] In this paper, we show that the nematic phase of a colloidal suspension of rod-shaped viruses provides an alternative to bicelles.

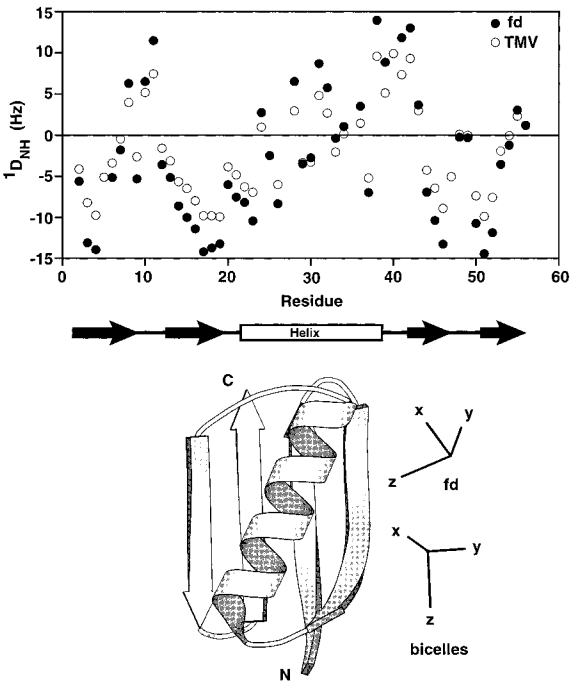

Figure 1. Plot of $^1D_{NH}$ measured at 750 MHz versus residue number for the protein G domain (∼0.5 mM) dissolved in the nematic phase of a colloidal suspension of *fd* (∼28 mg/mL) and TMV (∼50 mg/mL) at 20 °C in 50 mM sodium phosphate buffer pH 5.4. The secondary structure and a ribbon diagram of protein G[10] are shown below the figure, together with the axes for the alignment tensor obtained in the nematic phase of *fd* (top) and DMPC/DHPC bicelles (bottom).

Suspensions of charged, rod-shaped viruses, such as the filamentous baceriophage *fd* and tobacco mosaic virus (TMV), are known to undergo an isotropic−nematic phase transition at relatively low concentrations.[9] Figure 1 displays the residual one-bond N−H ($^1D_{NH}$) dipolar couplings for a ∼0.5 mM solution of streptococcal protein G (56 residues)[10] in colloidal suspensions of ∼28 mg/mL *fd* and ∼50 mg/mL TMV.[11] The values of $^1D_{NH}$ range from +15 to −15 Hz in the case of the *fd* suspension and +10 to −10 Hz in the case of the TMV suspension.

The general expression for the residual dipolar coupling $D_{AB}(\theta,\phi)$ between two directly coupled nuclei A and B is given by $D_a^{AB}\{(3\cos^2\theta - 1) + {}^3/_2R(\sin^2\theta\cos 2\phi)\}$ where D_a^{AB} and D_r^{AB} in units of hertz are the axial and rhombic components of the

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(8) Examples at the NIH where the bicelles have proved to be unstable include enzyme I, the enzyme I-HPR complex, the N-terminal domain of HIV-1 integrase coordinated to cadmium, SIV gp41 (G.M.C. and A.M.G., unpublished data), the Vα domain of a T-cell inhibitor (J. S. Hu and A. Bax, personal communication), and HIV protease (D. Torchia, personal communication).

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(10) Protein G (Gronenborn, A. M.; Filpula, D. R.; Essig, N. Z.; Achari, A.; Whitlow, M., Wingfield, P. T.; Clore, G. M. *Science* **1991**, *253*, 657−661) in a colloidal suspension of *fd* was prepared by adding the protein to a dilute (∼1−5 mg/mL) solution of *fd* in the appropriate buffer, followed by concentration to ∼0.5 mM protein and ∼25−28 mg/mL *fd*. Protein G in a colloidal suspension of TMV was prepared by adding a stock solution of TMV (210 mg/mL) in water to a protein solution in buffer to yield ∼0.5 mM in protein and ∼50 mg/mL TMV. Over a period of about 6 h, the TMV solution separated into a lower birefringent nematic phase and an upper isotropic phase. The upper isotropic phase was removed, and only the nematic phase was employed for NMR. As the pH of the solution approaches the pI of the virus particles (∼4.2 for *fd* and ∼3 for TMV), precipitation occurs. Once precipitation of *fd* has occurred, birefringence cannot be recovered, and the suspension remains isotropic, even though the *fd* particles can be resolubilized by raising the pH. Thus, solutions of *fd* should not be taken to pH values below 5.0. At this time, we have also employed suspensions of *fd* phage successfully for both enzyme I and the enzyme I-HPr complex, where previously a variety of lipid bicelles had proved to be unstable.

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Table 1. Alignment Tensor **D** and rms and *R* Factor between Observed and Calculated Residual $^1D_{NH}$ Dipolar Couplings for Protein G in the Nematic Phase of Colloidal Suspensions of *fd*, TMV, and Lipid Bicelles[a]

liquid crystal	α (deg)	β (deg)	γ (deg)	$^1D_a^{NH}$ (Hz)	D_r/D_a	rms (Hz)	*R* factor	no. of measured dipolar couplings
fd pH 5.4 (∼28 mg/mL)	70.6	−20.7	54.7	−7.7	0.66	1.45	0.13	48
fd pH 6.0 (∼25 mg/mL)	71.5	−20.8	54.7	−4.4	0.66	0.79	0.12	50
fd pH 8.0 (∼25 mg/mL)	72.0	−18.1	47.7	−6.9	0.66	1.25	0.12	44
TMV pH 5.4 (∼50 mg/mL)	70.6	−23.6	59.3	−5.2	0.61	0.87	0.12	51
bicelle pH 7.0 (50 mg/mL)	66.4	89.0	21.3	−9.7	0.23	1.47	0.12	49

[a] The parameters describing the magnitude ($^1D_a^{NH}$ and the rhombicity D_r/D_a) of the tensor **D** and its orientation (the Euler angles α, β, γ) relative to the coordinate frame of the 1.0 Å crystal structure[14] are obtained by least-squares optimization.[4f] The reported rms is the rms difference between the observed values and the values calculated on the basis of the known orientations of the N−H internuclear vectors in the X-ray structure. The *R* factor is the ratio of the measured rms to the expected rms if the N−H vectors were randomly distributed (i.e., a random coil). For an infinite number of measurements, the latter is given by $(2\langle D_{obs}^2\rangle)^{1/2}$ which is readily shown to be equal to $\{2D_a^2[4 + 3(D_r/D_a)^2]/5\}^{1/2}$.

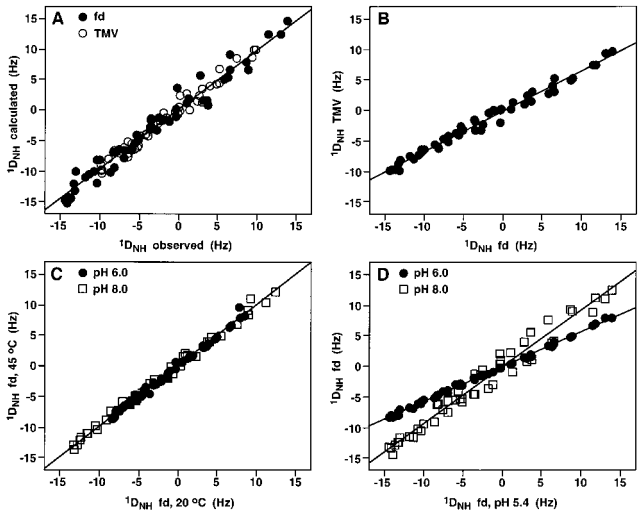

Figure 2. (A) Correlation between the values of $^1D_{NH}$ measured for protein G in the nematic phase of *fd* (∼28 mg/mL) and TMV (∼50 mg/mL) versus the values predicted on the basis of its 1.0 Å resolution crystal structure calculated using the magnitude and orientation of the alignment[14] tensor **D** given in Table 1. (B) Correlation between values of $^1D_{NH}$ measured for protein G (B) in *fd* (∼28 mg/mL) versus TMV (∼50 mg/mL) at 20 °C, pH 5.4, (C) in *fd* (∼25 mg/mL) at 20 °C versus 45 °C (for data at pH 6 and pH 8), (D) in *fd* (∼28 mg/mL) at pH 5.4 versus in *fd* (∼25 mg/mL) at pH 6 and 8 (at 20 °C). The samples at pH 5.4 and 6 were in 50 mM sodium phosphate, the sample at pH 8 in 10 mM Tris.

traceless second rank diagonal tensor **D**; *R* is the rhombicity defined by D_r^{AB}/D_a^{AB}; θ is the angle between the A−B interatomic vector and the *z* axis of the tensor; and ϕ is the angle which describes the position of the projection of the A−B interatomic vector on the *x*−*y* plane, relative to the *x* axis.[12] Figure 2A shows the correlation between observed values of $^1D_{NH}$ measured in the nematic phase of *fd* at pH 5.4 and the values calculated from the 1 Å crystal structure by optimization of the magnitude and orientation of the tensor **D**. Also shown are the correlations between observed $^1D_{NH}$ values obtained in different media (*fd* vs TMV; Figure 2B), at different temperatures (20 °C vs 45 °C, Figure 2C), and different pHs (pH 5.4 vs pH 6 and 8, Figure 2D). Although the magnitude of the axial component (D_a^{NH}) differs, indicating different degrees of alignment, the rhombicity and orientation of the tensor are similar in the *fd* and TMV nematic phases (Figure 2B, Table 1) and are unaffected by temperature (20 °C versus 45 °C, Figure 2C). pH, however, slightly perturbs the orientation of the tensor (Figure 2D, Table 1). A summary of the magnitude and orientation of the tensor for different experimental conditions is provided in Table 1. Indeed, we have found that stable nematic phases of *fd* and TMV in the presence of protein can be obtained at pH values above 5 and 4, respectively. Moreover, the samples of *fd* and TMV suspensions in the presence of protein remain birefringent over the entire temperature range (5 to 60 °C), thus being suitable for NMR studies on biological macromolecules.

As a control, the residual dipolar couplings of protein G in a 5% (wt/vol) liquid crystalline medium of 3:1 DMPC/DHPC were measured. Not only is the rhombicity very different (∼0.2 versus 0.6−0.66 for the *fd* and TMV suspensions), but the orientation of the tensor is also completely different (Figure 1, Table 1). Indeed, the angle between the *z* axis of the tensors in bicelles and in the *fd* and TMV suspensions differ by 78−83° (Figure 1). Exploiting these differences offers the possibility of refinement against two sets of dipolar couplings with very different tensor orientations and rhombicities, thereby increasing the information content afforded by the dipolar couplings.[6c]

In conclusion, we have shown that the nematic phase of a dilute colloidal suspension of rod-shaped viruses is highly suitable for obtaining moderate degrees of alignment of macromolecules for the purpose of measuring residual dipolar couplings. Other rod-shaped viruses of the filamentous phage family, such as Pf1 and M13, as well as other rigid, charged, rod-shaped macromolecular assemblies, such as flagellar filaments and F-actin,[13] known to form a nematic phase at dilute concentrations, may also be suitable for this purpose.

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(11) $^1D_{NH}$ values were obtained by taking the difference in the $^1J_{NH}$ scalar couplings measured in isotropic and nematic media. All of the measurements were carried out in duplicate, and the rms error in the measured $^1D_{NH}$ values ranges from 0.2 to 0.4 Hz. The ^{15}N and ^1H line widths of protein G are unaffected by the presense of the *fd* and TMV suspensions. The majority of spectra were recorded on a Bruker DMX750 spectrometer. Additional spectra were also recorded on a Bruker DMX600 spectrometer, and no difference in the values of $^1D_{NH}$ was observed between the data sets recorded at 750 and 600 MHz.

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