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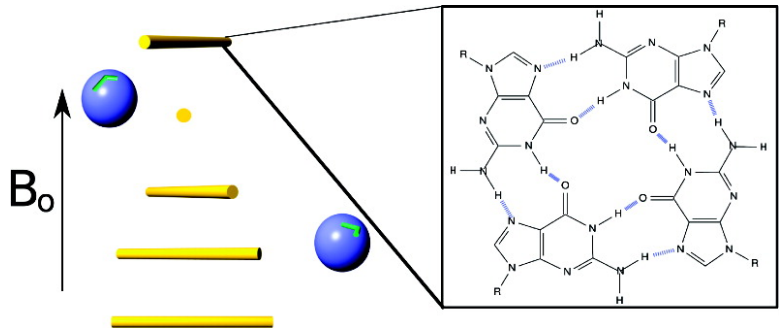

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Liquid Crystalline Phase of G-Tetrad DNA for NMR Study of Detergent-Solubilized Proteins

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Structure determination of membrane proteins remains an important but difficult research target. Solution NMR offers a number of experimentally accessible parameters that can aid in structural studies, including interproton distances derived from NOEs, torsion angles from J-couplings and chemical shift data, and vector orientations from residual dipolar couplings (RDCs).[1,2] RDCs constrain the orientations of internuclear vectors relative to a molecular axis system and therefore carry information that is inherently global in character. RDCs in solution result from an anisotropic distribution of molecular orientations in the sample. Such anisotropy can be introduced by adding a low volume fraction of nematogens to the aqueous solvent, which align in a liquid crystalline (LC) manner relative to an external strong magnetic field. Alternatively, weak macromolecular order can be induced by dissolving the protein in a highly hydrated, anisotropically compressed polyacrylamide gel,[3,4] or use of a covalently attached moiety that can chelate paramagnetic ions.[5]

LC media commonly used for water-soluble proteins, such as bicelles, Pf1, and polyethylene-glycol mixtures are incompatible with detergents and lipids. Detergents can be used with acrylamide gels,[6,7] but such gels can decrease the rotational tumbling rate, especially for larger systems. [3,4] Recently, Douglas et al.[8] reported the first detergent-compatible liquid crystal suitable for the NMR study of membrane proteins: a DNA-nanotube consisting of a 7308-basepair "scaffold" and 42-basepair "staple" DNA molecules. They demonstrated alignment and measurement of RDCs for the ζ chain of the T-cell receptor complex in 150 mM dodecylphosphocholine (DPC) and 30 mM sodium dodecylsulfate (SDS). However, production of the required tens of milligrams of nanotubes, needed for a protein NMR study, remains a very labor-intensive and expensive process.

Inspired by the work on these DNA-based nanotubes, we have exploited the inertness of nucleic acid secondary structure to many detergents to explore the utility of simpler nucleic acid-based liquid crystals. One such particularly suitable liquid crystal consists simply of the dinucleotide 2′-deoxyguanylyl-(3′,5′)-2′-deoxyguanosine, d(GpG). Guanosine mono-, di-, and larger oligonucleotides form G-tetrad structures, where hydrogen bonds link four G bases in a C_4-symmetric planar arrangement, with cations on the C_4 axis coordinating the C_6 carbonyl groups of G.[9,10]

Indeed, above ca. 10 mg/ml d(GpG) forms a cholesteric LC phase that aligns in a magnetic field,[11] whereas at much higher concentrations it adopts a hexagonal LC phase.[12] As has long been known, stability of the G-tetrameric planar arrangement depends on the size of the coordinating monovalent cations, with K^+ yielding the highest stability and a high nematic-to-isotropic transition temperature of ca. 40 °C for d(GpG). K-d(GpG) therefore is well suited for the NMR study of larger proteins, where measurement at or above room temperature is preferred in order to minimize microscopic solvent viscosity and thereby maximize spectral resolution.

Table 1. Alignment Tensor Parameters in Liquid Crystalline Media for U-{^{13}C,^{15}N}-[K19E,D40N,V42E]-GB3[a] [15,22]

LC medium	D_a (Hz)	R	tensor orientation[b]	Q-factor
Pf1	−7.12	0.321	(154°, 90°, 220°)	0.083
K-dGpG	8.30	0.434	(156°, 90°, 232°)	0.096

[a] Tensor parameters based on 48 NH RDC measurements. Data recorded in 25 mM K_2HPO_4 pH 7.4, 35 mM KCl, 22 mg/ml dGpG [Sigma] at 25 °C. The Pf1 sample contained 22 mg/ml Pf1 bacteriophage [Asla Biotech], 25 mM NaH_2PO_4, pH 6.7, 50 mM NaCl. [b] Tensor orientation expressed as the Rose convention Euler angles between the GB3 molecular frame (2OED) and the alignment frame.

Stacked columns of G-tetramers, which have a diameter of ∼2.5 nm (vs 6.5 nm for Pf1) carry a high net negative charge of ca. 0.4 e/nm^{-2} (∼0.5 e/nm^{-2} for Pf1[13]). Their similarity in shape and charge to Pf1 suggests that alignment tensors for proteins compatible with both types of liquid crystals will be directly related to one another.[14] We first demonstrate this similarity for a mutant of GB3, previously extensively characterized by RDCs.[15,16] A small region of the ^{1}H-coupled ^{1}H−^{15}N HSQC NMR spectrum is included as Supporting Information, and the alignment tensors in K-d(GpG) and Pf1 media are compared in Table 1. The normalized scalar product[17] of the protein alignment tensors in d(GpG) and Pf1 is −0.983, confirming that protein alignments in these two media are simply related by a scale factor. The opposite sign of the two alignments results from the different orientations of the rod-shaped particles in the magnetic field. The magnetic susceptibility anisotropy of Pf1 is dominated by that of its major coat protein helices, causing the long axis of the phage filament to align parallel with the applied magnetic field.[18] K-d(GpG) alignment is dominated by the diamagnetic purine bases, which have lowest energy when the external magnetic field is parallel to the plane of the base, orienting the columns orthogonal to the field.

K-d(GpG) maintains a liquid crystalline phase in the presence of DPC detergent. Furthermore, the liquid crystalline phase is maintained at moderately acidic pH (Figure 1) as well as in the presence of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPPC) detergent or the more membrane-like mixed micelles,[19] often referred to as small bicelles (data not shown). Simply dissolving the K-d(GpG) overnight, followed by addition of KCl, results in a sample with a stable residual quadrupole coupling (RQC) for the ^{2}H lock signal (Figure 1). The transition temperature to the isotropic phase depends on the d(GpG) concentration and depreciates by ∼10 °C when the concentration is lowered from 20 to 10 mg/ml; the threshold for liquid crystal formation increases to ca. 22 mg/mL when the DHPC concentration reaches 200 mM (Supporting Information).

Figure 2 shows $^1H^N$-^{15}N IPAP-HSQC[20] and $J_{C\alpha H\alpha}$-coupled HNCA spectra for the U-{^{13}C,^{15}N-gly,ala} hemagglutinin fusion peptide (HAfp) solubilized in DPC, in the absence and presence of K-d(GpG). Partial alignment of the fusion peptide in DPC

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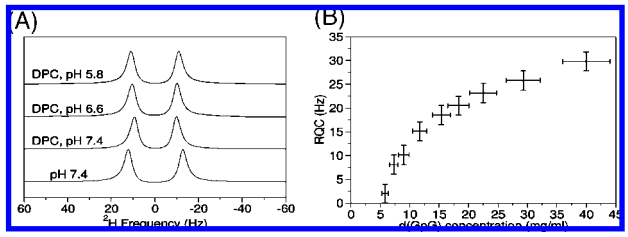

Figure 1. Residual ^2H_2O quadrupole coupling in the presence of d(GpG). (A) Spectra of ^2H_2O with and without DPC and (B) the dependence of the ^2H_2O RQC on the concentration of d(GpG). (A) RQCs are 21.9 Hz (DPC, pH 5.8), 20.6 Hz (DPC, pH 6.6), 21.9 Hz (DPC, pH 7.4) and 25.0 Hz (pH7.4). The pH titration was carried out on a sample of 3 mM U-{^{13}C,^{15}N-gly,ala}-HAfp and 28 mg/ml d(GpG), 23 mM K_2HPO_4, 81 mM DPC on a Bruker 500 MHz DMX NMR spectrometer at 32 °C. See footnote to Table 1 for sample details on the sample without DPC. (B) Concentration dependence of the ^2H_2O residual quadrupolar coupling.

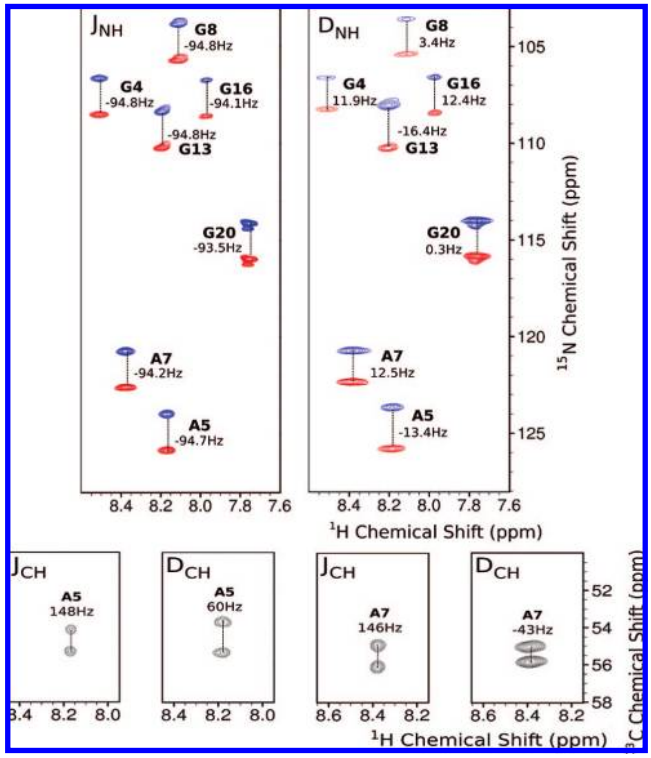

Figure 2. $^1H-^{15}N$ IPAP-HSQC[20] and $J_{C\alpha H\alpha}$-coupled HNCA spectra for 2.1 mM U-{^{13}C,^{15}N-gly,ala}-HAfp [Biopeptide, sequence: GLFGAIAG FIENGWEG MIDG-OH] in 81 mM DPC [Anatrace], 23 mM K_2HPO_4, pH 7.4. J_{NH} and J_{CH} splittings are shown for the isotropic sample. RDCs (D_{NH} and D_{CH}), obtained from the change in splitting are shown for the spectra recorded after addition of 28 mg/ml d(GpG). Spectra containing the upfield (blue) and downfield (red) ^{15}N-{^1H} doublet components of the $^1H-^{15}N$ IPAP-HSQC spectra are superimposed. Measurements conducted on a Bruker 500 MHz DMX NMR spectrometer at 32 °C.

micelles adds a RDC to the amide $^1J_{HN}$ and $^1J_{C\alpha H\alpha}$ splittings.[2] Studies of uniformly enriched fusion domain are currently underway to obtain a more detailed characterization of the pH-induced structural transition, which triggers fusion of the virus with host cell membranes.[21]

G-tetrad formation previously has been studied extensively by a range of physical and biophysical chemistry methods. Although its biological role has been subject to extensive debate, G-tetrads now are increasingly recognized as a critical element in the telomeric sequences protecting the termini of chromosomes.[9] The high stability of stacked G-tetrad polymers also gives them a high persistence length, making them suitable for liquid crystal formation at the dilute concentrations required for biomolecular NMR. Their compatibility with a range of commonly used mild surfactants offers new opportunities for the structural study of detergent-solubilized biological macromolecules. Like Pf1, the negative charge of K-d(GpG) makes it best suited for the study of negatively charged macromolecules. However, with peptide-nucleic-acid chemistry well established, adjustment of the charge characteristics of G-tetrad-based liquid crystals is likely to be feasible too, a subject we are currently exploring.

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Supporting Information Available: Protocol for NMR sample preparation; plots of (A) GB3 $^1D_{NH}$ RDCs in Pf1 vs d(GpG), (B) a region of the IPAP-HSQC $^1H-^{15}N$ spectrum of GB3 in d(GpG), ^2H RQC as a function of temperature at 100 and 214 mM DPC. This material is available free of charge via the Internet at http://pubs.acs.org.

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