Alignment of Biopolymers in Strained Gels: A New Way To Create Detectable Dipole−Dipole Couplings in High-Resolution Biomolecular NMR

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An important recent development in high-resolution nuclear magnetic resonance (NMR) spectroscopy has been the demonstration that orientation-dependent nuclear spin interactions, such as nuclear magnetic dipole−dipole couplings, anisotropic chemical shifts, and nuclear electric quadrupole couplings, can provide useful information about the structures of biological macromolecules in aqueous solutions when the macromolecules are weakly aligned.[1−6] Methods for creating weakly aligned states include direct magnetic alignment,[1−4] alignment in liquid crystalline mesophases,[5−14] and alignment in electric fields.[15−17] Here we describe a new approach to weak alignment of biopolymers for high-resolution NMR and possibly other forms of spectroscopy.[18] We show that a protein can be aligned by dissolution in an anisotropic aqueous polymeric gel. Anisotropy is induced by straining (i.e., compressing or stretching) the gel away from an initial isotropic state. This approach is conceptually related to earlier experiments by Deloche and Samulski[19] in which alignment of small organic molecules such as benzene and chloroform dissolved in rubbery synthetic polymers such as polyisoprene was detected in the form of quadrupole splittings in ^{2}H NMR spectra when the polymer was stretched. Our use of an aqueous gel in place of a rubbery polymer allows high-resolution NMR measurements on biopolymers that are soluble and structured in aqueous solutions.

Experiments were performed on uniformly ^{15}N-labeled samples of the B1 immunoglobulin-binding domain of streptococcal protein G (protein G/B1), a small protein whose structure has been determined by high-resolution NMR[20] and by X-ray crystallography,[21] dissolved in cross-linked polyacrylamide gels. Our clone produced an N-formylated, 57-residue variant of protein G/B1,[22] with an additional aspartate residue inserted between the first and second residues of the amino acid sequence in refs 20 and 21. Gels were cast at room temperature in the form of rods with a 3.6 mm diameter, approximately 0.5 mm less than the inner diameter of a standard 5 mm NMR tube, using a solution of 7.66% w/v acrylamide and 0.40% w/v *N,N′*-methylenebisacrylamide with polymerization initiated by 0.05% w/v ammonium persulfate and 0.05% v/v *N,N,N′,N′*-tetramethylethylenediamine. Nominal pore size was roughly 20 nm.[23] After gelation and washing with water, gel rods were cut to 40 mm length and placed in the NMR tube between Aurum spacers (Doty susceptibility plugs, Wilmad). Protein molecules entered the gel by diffusion from an exterior solution. Upon application of pressure to the top spacer with a fiberglass rod held in place by the NMR tube cap, gels compressed in length to 30 mm and expanded in diameter to fill the tube. Compressed gels exhibited optical birefringence. NMR experiments were carried out on a Varian/Chemagnetics Infinity-400 spectrometer operating at a ^{1}H NMR frequency of 400.95 MHz. Compression as described creates a nominally uniaxial strain with unique axis parallel to the external magnetic field, although precise alignment with the field is not necessary. Two-dimensional (2D) ^{15}N/^{1}H heteronuclear single quantum coherence (HSQC) spectra were recorded without ^{1}H decoupling in the ^{15}N dimension and both with and without gel compression, at protein concentrations of approximately 1 mM, temperatures between 5 and 45 °C, pH values between 2.0 and 8.5, and NaCl concentrations between approximately 0 and 200 mM.

Figure 1a shows one-dimensional slices of undecoupled HSQC spectra that illustrate the effects of gel compression. In the uncompressed state, doublet splittings of 92−95 Hz due to one-bond ^{1}H−^{15}N scalar couplings were observed. In the compressed state, the splittings changed by −12 to +9 Hz. We attribute these changes to small, time-averaged ^{1}H−^{15}N dipole−dipole couplings induced by weak dynamic alignment of protein molecules in the strained gel. Similar spectra were obtained under conditions listed in Table 1. Scalar couplings in unstrained gels were the same as in pure aqueous solution. Inhomogeneous broadening of protein ^{1}H NMR lines by approximately 4 Hz was observed in gels and attributed to imperfect shimming and nonuniform gel density.

Experimental dipole−dipole splittings D_{exp} were extracted by comparison of compressed and uncompressed doublet splittings, using peak fitting software within the nmrPipe software package.[24] Calculated dipole−dipole splittings D_{fit} for each backbone amide site were determined by the equation $D_{\mathrm{fit}} = A \sin^2\theta \cos^2\theta + B \sin^2\theta \sin^2\phi + C \cos^2\theta$ appropriate for uniaxial strain, where A, B, and C represent products of the principal values of the traceless, second-rank alignment tensor with the ^{1}H−^{15}N dipole−dipole coupling constant, and θ and ϕ specify the direction of the N−H bond in the principal axis system of the alignment tensor. Atomic coordinates for protein G/B1 in crystalline form were obtained from PDB file 1pga. Hydrogen atom coordinates were calculated by the program MOLMOL.[25] Nearly identical results were obtained using PDB files 1pgb and 3gb1. The orientation of the alignment tensor relative to the crystal structure coordinate system

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Table 1. Sample Conditions and Best-Fit Parameters Describing the Alignment Tensor for Protein G/B1 in a Compressed, Cross-linked Polyacrylamide Gel[a]

T (°C)	pH	[NaCl] (mM)	A, B, C (Hz)	α, β, γ (deg)	no. of ^{1}H-^{15}N couplings analyzed[b]
28	4.5	0	-9.9 ± 0.6, -0.8 ± 1.0, 10.7 ± 0.8	173 ± 5, 111 ± 5, 140 ± 5	47
45	4.5	0	-9.9 ± 0.6, 0.9 ± 0.5, 9.0 ± 0.4	170 ± 5, 110 ± 4, 140 ± 4	44
5	4.5	0	-2.8 ± 0.6, -0.9 ± 0.7, 3.7 ± 1.0	171 ± 12, 105 ± 10, 149 ± 20	38
28	2.0	0	-16.0 ± 0.6, 4.7 ± 0.7, 11.3 ± 0.7	155 ± 4, 94 ± 6, 136 ± 4	16
28	8.5	0	-8.8 ± 0.5, -2.0 ± 1.0, 10.8 ± 1.5	182 ± 5, 112 ± 4, 147 ± 8	42
28	4.5	200	-11.1 ± 0.5, 2.6 ± 0.4, 8.5 ± 0.6	168 ± 6, 115 ± 5, 138 ± 5	26

[a] Error limits represent variations in individual parameters that produce a change in χ^2 of two units, assuming a 2 Hz uncertainty in experimental dipole−dipole couplings. [b] All couplings that were well-resolved in the undecoupled HSQC spectrum and could be assigned unambiguously by comparison with spectra at pH 4.5 and 28 °C were analyzed.

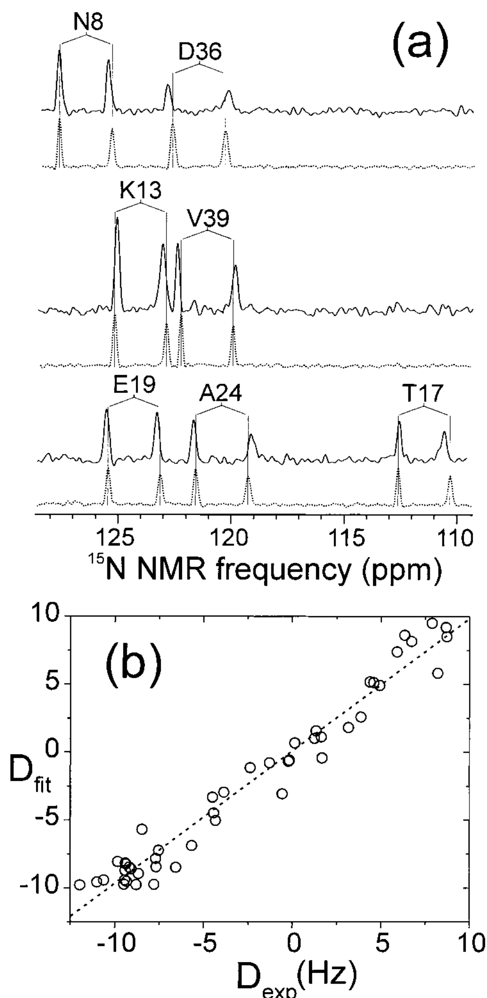

Figure 1. (a) One-dimensional slices of undecoupled two-dimensional ^{1}H/^{15}N HSQC spectra of uniformly ^{15}N-labeled protein G/B1 in a cross-linked polyacrylamide gel at 28 °C, pH 4.5, and approximately 0 mM NaCl, taken at ^{1}H chemical shifts of 8.90, 8.12, and 8.04 ppm (top to bottom). Each backbone amide site appears as a doublet. Residue assignments from two-dimensional heteronuclear and homonuclear experiments are indicated. Doublet splittings observed when the gel is strained by compression to 75% of its initial length (solid lines) either increase (D36, V39, A24) or decrease (N8, K13, E19, T17) relative to splittings in the uncompressed state (dotted lines). The changes in splittings are ^{1}H−^{15}N dipole−dipole couplings. (b) Correlation of experimental and fitted dipole−dipole couplings. Fitting is based on the known protein structure as described in the text. The dashed line is a linear regression, with slope 0.98 ± 0.03, intercept 0.11 ± 0.20, and correlation coefficient $R = 0.981$.

was specified by Euler angles α, β, and γ. The five fitting parameters α, β, γ, C, and $\eta = (B - A)/C$ were adjusted to minimize the total squared deviation between D_{exp} and D_{fit} values. The agreement between D_{exp} and the resulting D_{fit} values is shown in Figure 1b for data acquired at 28 °C, pH 4.5, and approximately 0 mM salt. Figure 2 shows the orientation of the alignment tensor relative to the protein structure obtained from these data. Given that the ^{1}H−^{15}N scalar couplings are negative, the observation that C is positive (i.e., splittings increase at $\theta = 0$) implies that the corresponding principal axis tends to align perpendicular to the unique strain axis and the magnetic field. Values of the fitting parameters obtained under various experimental conditions are given in Table 1. Strong dependence of the orientation of the alignment tensor on sample conditions is not observed. We attribute the smaller alignment at 5 °C to reduction in strain due to thermal contraction of the gel within the NMR tube. The moment of inertia tensor calculated from PDB file 1pga has orientation $\alpha,\beta,\gamma = 179°,125°,121°$, quite similar to the orientation of the alignment tensor. As shown in Figure 2, the alignment tensor principal axes appear to conform to the shape of the protein, suggesting a predominantly steric mode of alignment.

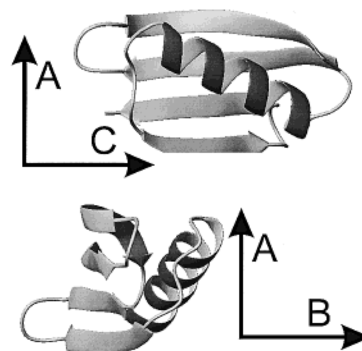

Figure 2. Orientation of alignment tensor principal axes for protein G/B1, derived from fitting dipole−dipole couplings in a strained gel, relative to the protein structure. The notation corresponds to the principal values in Table 1. Axes B and C are perpendicular to the page in the top and bottom views, respectively. (Figure created with MOLMOL.[25])

We refer to this molecular alignment method by the acronym SAG (strain-induced alignment in a gel). Advantageous features of SAG include its effectiveness over wide ranges of temperature, pH, and ionic strength and its likely compatibility with a wide range of biopolymers, irrespective of surface charge or other properties. The degree of alignment by SAG can be varied by varying the amount of strain, the gel density, and possibly the gel composition. Biopolymer samples can be recovered from the gel by diffusive dilution into an exterior solution. The direction of molecular alignment by SAG is dictated by the direction of strain, not by the magnetic field. In the case of a biaxially strained gel (i.e., a nonaxially symmetric medium), independent sets of structural constraints could be obtained from a single sample by performing measurements at several different orientations with respect to the magnetic field.

Supporting Information Available: HSQC spectra of protein G/B1 in pure aqueous solution, unstrained polyacrylamide gel, and strained gel (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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