Modulation of the Alignment Tensor of Macromolecules Dissolved in a Dilute Liquid Crystalline Medium

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Dipolar couplings measured for pairs of nuclei constrain the possible orientations of internuclear vectors relative to the molecule's alignment tensor.[1−3] For macromolecules such as proteins and nucleic acids, the required small degree of alignment with the magnetic field can result from the molecules' own magnetic susceptibility anisotropy[2−4] or can be induced by the use of a liquid crystalline medium.[1] Provided the alignment is sufficiently weak, only directly bonded pairs of atoms give rise to a measurable dipolar coupling, which manifests itself as a change in the corresponding scalar interaction. The spectral simplicity of the high-resolution NMR spectrum is therefore retained, and measurement of dipolar couplings is straightforward. The most commonly used liquid crystalline medium for inducing macromolecular alignment consists of large, disk-shaped phospholipid particles, often referred to as bicelles.[5,6] The volume fraction of phospholipid particles can be adjusted to obtain the desired degree of alignment.[7]

For the case where the diagonalized alignment tensor is axially symmetric, and the internuclear distance is accurately known, each dipolar coupling restrains the position of the corresponding internuclear vector to a cone about the unique axis of the alignment tensor. For the general case, where the alignment tensor is asymmetric, the cone is distorted and "taco-shaped".[8] Because the direction of a second rank tensor interaction cannot be distinguished from its inverse, the dipolar coupling actually defines two cones of possible bond vector orientations, in opposing directions. Although such information is clearly very useful in determining macromolecular structures more accurately,[4,6] the continuum of possible orientations makes it difficult to determine local geometry in the absence of other information. Here we demonstrate that the degeneracy can be lifted by recording a second set of dipolar couplings under conditions where the orientation and/or rhombicity of the alignment tensor is altered. Three different approaches are shown to be successful: (1) addition of an unstructured, so-called His-tag peptide at the C-terminus of the protein, (2) changing the sample pH, and (3) changing the net charge of the bicelles.

For recombinant proteins, it is frequently possible to add a short unstructured peptide at either the N- or C-terminus. Although such an additional peptide fragment does not alter the structure of the globular domain, it adds a flexible "tail" to the protein which affects the molecule's alignment tensor. For example, a so-called His-tag sequence frequently is added to a heterologously expressed protein to facilitate its purification. Histidine residues have a pK of ∼7, and their net charge can be altered by lowering the pH from 7.5 to 5. If there is a small amount of net charge on the surface of the bicelles, this will change the very weak electrostatic attraction/repulsion between the protein and the bicelle, thereby altering the alignment tensor. Finally, we demonstrate that deliberately adding a net charge to the bicelles, recently shown to increase the stability of the liquid crystalline phase at low volume fractions,[9] also can be used to alter the alignment tensor, in either the presence or absence of the His-tag sequence.

Experiments are carried out for samples of uniformly $^{13}C/^{15}N$-enriched ubiquitin, either with an additional SHHHHHH peptide at its C-terminus (Martek Biosciences, Columbia, MD) or without (VLI Research, Southeastern, PA). Samples were ∼0.7 mM, in 93% H_2O, 7% D_2O, containing 4−5% (w/v) bicelles (consisting of a 3:1 molar ratio of DMPC to DHPC).[5,6] As a second test, measurements were conducted for a sample of uniformly ^{15}N-labeled aprotinin (often referred to as BPTI) (0.2 mM in 4−5% (w/v) bicelles).

The dipolar coupling between two nuclei, A and B, in a solute macromolecule of fixed shape is related to the traceless alignment tensor according to

$$D^{AB} = \sum_{i=x,y,z} -S(\mu_o h/8\pi^3)\gamma_A\gamma_B\langle r_{AB}{}^{-3}\rangle \cos^2\phi_i A_{ii} \quad (1)$$

where ϕ_i is the angle between the A−B bond vector and the A_{ii} principal axis of the diagonalized traceless molecular alignment tensor, γ_A and γ_B are the gyromagnetic ratios of the two nuclei, $\langle r_{AB}{}^{-3}\rangle$ is the vibrationally averaged inverse cube of the distance between the two nuclei, and S is the Lipari−Szabo generalized order parameter[10] which accounts for the effect of rapid internal motions.[3,10,11] For the polypeptide backbone, S is mostly quite uniform and effectively only results in a small scaling of **A**. Below we assume $S = 1$. If a protein contains highly flexible residues, these are typically easily recognized by their relaxation characteristics and these residues must be excluded.

The magnitude (two unknowns) and orientation of **A** relative to the molecular frame (three unknowns) are readily obtained from a fitting program which minimizes the differences between measured dipolar couplings and those predicted by eq 1 on the basis of the molecule's known shape.[3] Because the number of measured dipolar couplings far exceeds the number of unknowns, the problem is overdetermined and a Powell minimization routine is used to calculate **A**. Note that if the structure of the protein is not known, the magnitude of **A** can be obtained from the distribution of measured dipolar couplings[12] and its orientation is left "floating" during the structure calculation.[13]

Figure 1 compares the one-bond $^{15}N-^{1}H$ dipolar couplings measured in native ubiquitin in regular bicelles and in bicelles

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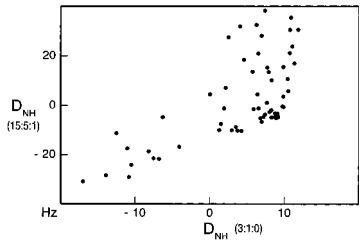

Figure 1. Plot of $^{15}N-^{1}H$ one-bond dipolar couplings (D_{NH}) measured in human ubiquitin in positively charged bicelles (DMPC:DHPC:CTAB = 15:5:1; 5% w/v) vs values measured in undoped bicelles (DMPC:DHPC:CTAB = 3:1:0; 5% w/v). The random measurement uncertainty in D_{NH}, based on reproducibility of consecutive measurements, is 0.2 Hz.

Table 1. Alignment Tensor of Ubiquitin in Several Liquid Crystalline Phases[a]

M:H:C:MA[b]	conc[c] (%)	α	β	γ	$10^4 A_{zz}$	$10^4 A_{yy}$	$10^4 A_{xx}$
30:10:0:1	5	33.2	41.4	49.1	−4.69	2.90	1.79
30:10:0:0	5	33.1	41.3	50.7	−5.31	3.29	2.02
30:10:1:0	5	30.9	29.9	20.3	−7.15	6.02	1.13
30:10:2:0	4.7	31.2	24.9	22.9	−10.64	11.62	0.98

[a] Using bicelles in 93% H_2O, 7% D_2O, at pH 6.75, 310 K. The Euler angles α, β, and γ define the alignment tensor relative to the coordinate frame of the 1.8-Å X-ray structure.[16] [b] Molar ratios of lipids. M = [DMPC]; H = [DHPC]; C = [CTAB]; MA = [myristic acid]. [c] Weight by volume for M + H + C + MA in water.

that are positively charged by addition of a small mole fraction of cetyltrimethylammonium bromide (CTAB). Upon addition of positive charge to the bicelles, the rhombicity of the alignment tensor (and the total degree of alignment) increases and at a 1:15 ratio of CTAB to DMPC the A_{11} component becomes smaller in magnitude than A_{22}. This corresponds to a "flip" of the axis system when **A** is decomposed into an axial and rhombic component. Changes in dipolar couplings between the two samples are up to 2 orders of magnitude larger than the uncertainties in the measurements. The magnitude and orientation of **A** for four slightly different liquid crystals are listed in Table 1.

For aprotinin, addition of positive charge to the bicelles decreases the magnitude and rhombicity of the alignment tensor (Supporting Information) and doping with negative charge, by the addition of myristic acid (in a 1:30 molar ratio relative to DMPC), resulted in precipitation of the bicelles. We therefore were unable to check whether the aprotinin alignment increases further upon addition of negative charge.

Figure 2 shows the possible orientations of the N−H bond vector of residue Gln^{40} in ubiquitin in pure bicelles and in the presence of positive doping of the bicelles with CTAB (DMPC:DHPC:CTAB = 30:10:1). The effect of an (exaggerated) ±1 Hz uncertainty in the measurement of $^{1}D_{NH}$ is also shown. Clearly the allowed orientations for the N−H bond vector, defined by the intersections of the two taco-shaped rings (each corresponding to the intersect of the allowed cone of N−H orientations and a sphere), are far more restrictive than either cone alone.

The uniqueness of the dipolar coupling information obtained with a second, modified alignment tensor depends on how different the two alignment tensors are. Increasing the magnitude but not changing either the A_{11}:A_{22}:A_{33} ratio or the orientation of **A** relative to the liquid crystal director does not alter the cone of allowed vector orientations and, therefore, would not provide new, independent information. The uniqueness of the dipolar coupling information obtained from two separate measurements is related to the angle, θ, at which the two cones of allowed bond vector orientations intersect one another (Figure 2). These angles are listed in the Supporting Information. For ubiquitin N−H vectors in the absence or presence of CTAB (DMPC:DHPC:CTAB = 30:10:1), the average absolute value of these angles is 30°, with only 17 out of 62 residues having an intersect angle smaller than 10°. Similarly, for aprotinin in the absence or presence of CTAB (15:5:1), an average intersect angle of 25° is found, with only 9 out of 45 amides having an intersect angle less than 10°.

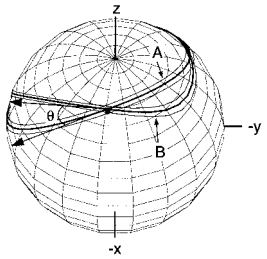

Figure 2. Orientations of the Gln^{40} N−H vector compatible with the measured dipolar couplings in undoped bicelles (band A) and in positively charged bicelles (band B). Orientations are given in the coordinate frame of the X-ray crystal structure.[16] The heavy lines correspond to the measured dipolar couplings; thinner lines correspond to orientations if D_{NH} is increased or decreased by 1 Hz. The angle θ at which the two distorted cones intersect equals 29°. The solid dot marks the orientation of the N−H vector in the crystal structure, with hydrogen positions model-built with the XPLOR program, assuming H^N falls exactly in the C′−N−C^α plane.

Although somewhat smaller in magnitude compared to the addition of CTAB to the liquid crystal, considerable changes in ubiquitin's alignment tensor are obtained by using a protein with an additional His-tag sequence at its C-terminus. Moreover, for this form of the protein, increasing the pH from 5 to 7.5 also leads to significant changes (Supporting Information) whereas for regular ubiquitin it does not (data not shown). In comparing the dipolar couplings measured in human ubiquitin and in the form that includes the C-terminal His-tag peptide, it is important to note that the chemical shifts for the ordered regions of the two proteins (residues 1−71) are essentially indistinguishable from one another, indicating identical structures for this region.

In essence, the ability to modulate the molecular alignment tensor by adding a nonperturbing tail, by titrating the charges of surface residues, or by adding positive or negative charges to the bicelles provides a "stereoview" of the protein. It is likely that using different, nonbicelle-type liquid crystals will yet provide another means for altering the alignment tensor. Simultaneous measurement of the three D_{NH}, $D_{C'N}$, and $D_{C'HN}$ dipolar couplings for each peptide group is readily possible in perdeuterated proteins of up to 30 kDa[14] and possibly larger.[15] With two different alignment tensors, the orientation of each peptide plane is then defined with only 2-fold ambiguity (a 180° rotation about the normal to the peptide plane) and it may become possible to determine a protein structure from these dipolar couplings using only a minimum of additional NOE or modeling information.

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Supporting Information Available: Two tables listing the alignment tensor information for aprotinin and His-tag ubiquitin; two tables with intersect angles of N−H cones for ubiquitin and aprotinin (3 pages, print/PDF). See any current masthead for ordering and Web access instructions.

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