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Local Dynamic Amplitudes on the Protein Backbone from Dipolar Couplings: Toward the Elucidation of Slower Motions in Biomolecules

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NMR spectroscopy occupies a unique position among biophysical techniques due to its particular sensitivity to intramolecular motions.[1] Rapid movements, up to the range of the characteristic rotational correlation time of the molecule (around 10 ns for medium-sized proteins in aqueous solution) are now routinely studied using ^{15}N spin relaxation. However while slower time scales (up to millisecond) can be detected, using for example relaxation dispersion methods,[2] routine measurement of these functionally important motions remains elusive.

Residual dipolar couplings (RDCs) report on averages over longer time scales (up to the millisecond range) and therefore encode key information for understanding protein motions in the submicro- to millisecond range.[3] Recent studies of conformational averaging from RDCs have used "model-free" approaches to characterize the reorientational properties of individual N−^{N}H vectors.[4] A drawback of these elegant methods is the practically stringent requirement of finding multiple (>5) alignment media that induce a sufficiently different residual orientation of the molecule relative to the magnetic field (the alignment tensor). It is therefore of interest to develop procedures for detecting conformational averaging from RDCs measured using fewer alignment media.

We have recently demonstrated the utility of applying a simple geometric model of peptide reorientation (Gaussian axial fluctuation or GAF)[5] to interpret conformational averaging of RDCs.[6] This study detected average amplitudes of anisotropic peptide plane dynamics of around 15° in secondary structural elements from different proteins using data from single alignment media. Here we apply this approach to the study of local peptide dynamics along the protein backbone.

In the presence of anisotropic peptide plane dynamics, N−^{N}H RDC averaging depends on the average orientation of the plane with respect to each tensor and on the amplitude of the conformational sampling (σ).[6] If the orientation and alignment tensors are known (from a structural model), motional amplitudes can then be determined for each site. Definition of this parameter may be ill-defined in the presence of a single tensor, so that for a robust analysis of the data-fitting procedure alignment media inducing significantly different tensors are required. We therefore combine RDC data from m alignment media to extract local motional amplitudes (σ_i) at each site i throughout the protein, by minimizing the function:[7]

$$\chi_i^2 = \sum_m (\{\langle D^i_{m,\mathrm{calc}}\rangle_{\mathrm{GAF}} - D^i_{m,\mathrm{exp}}\}^2/(\delta^i_m)^2) \qquad (1)$$

We have initially applied this approach to a set of five independent data sets recently measured from the third IgG-binding domain from protein G.[8] The non-dynamically averaged alignment tensor parameters were determined using $^{\alpha}$C−C′ RDC in comparison to the known structure, as this coupling has been identified as the least sensitive peptide plane RDC to GAF-like motions.[6] The available N−^{N}H RDCs for each site were then fit to the crystal structure[9] using eq 1 to optimize σ_i with respect to these tensors. Model acceptance is based on analysis of the quality of the fit.[10] There is a clear statistical improvement in the reproduction of the experimental data using this simple dynamic model, and in general the GAF motional model accounts for a large amount of the discrepancy between RDC calculated from the static model and the experimental data (total χ^2 for the 27 sites using the GAF model is 318, while for the static model this is 1267).

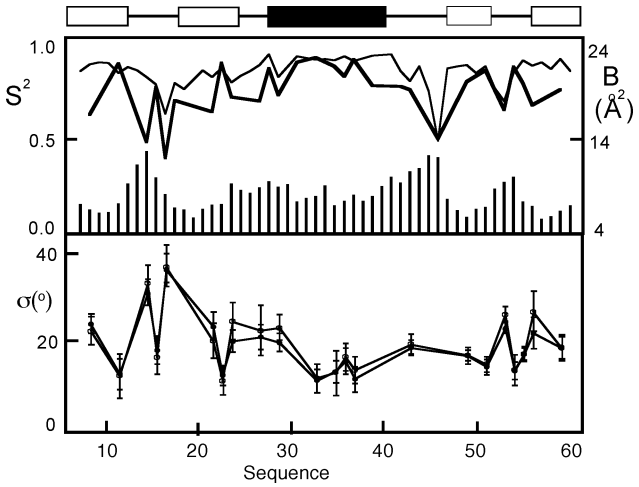

Figure 1. Dynamic parameters from RDC measured in protein G. (Top) Order parameters derived from GAF analysis of RDC, (thick line) compared to S^2 derived from ^{15}N relaxation (thin line) and B-factors from the crystal study (histogram). The position of the α-helix is indicated in black, and β-sheets, in white. (Bottom) GAF amplitudes derived using all five data sets (open circles) and the two most independent (filled circles). Errors are estimated from noise-based Monte Carlo simulations, assuming an error of 1 Hz.

Comparison with analogous dynamic amplitudes derived from ^{15}N relaxation, reporting on motions occurring on the pico- to nanosecond time scales, should allow the identification of sites undergoing longer time-scale motions. We have therefore translated the amplitude σ into an order parameter S^2_{RDC}[5a] and compared this with ^{15}N relaxation-derived order parameters[11] (Figure 1a). Similar amplitude motions are found in the central α-helix of the protein, with higher-order parameters characteristic of a compact, relatively rigid structural domain. The most dynamic regions are clustered around the protruding loop between strands 1 and 2 of the β-sheet, while the fourth is located at the same extremity of the β-sheet in the loop preceding strand 3. While the location of these larger-amplitude dynamics corresponds to that found from ^{15}N relaxation, for certain positions the amplitude is significantly greater. This is perhaps not surprising, considering the longer time scale over which averaging is occurring. Importantly, no order parameters are

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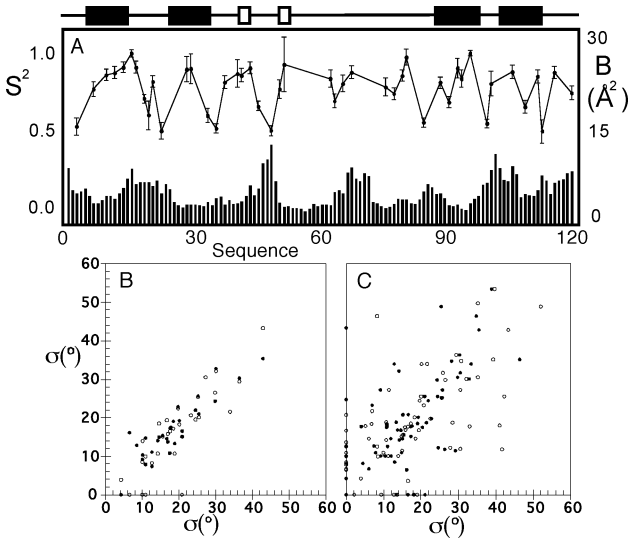

Figure 2. Dynamic parameters from RDC measured in lysozyme. (A) Order parameters derived from GAF analysis of RDC (line) compared to *B*-factors from X-ray[14] (histogram). Helices are indicated in black. No fits between residues 121−129 were accepted. (B,C) Comparison of GAF amplitudes derived using 0.9 Å (*x*-axis) and 1.5 Å (filled circles) and 2.1 Å (open circles) structures (*y*-axes) applying selection criteria (B) and for all fits (C).

measurably higher than those derived from ^{15}N relaxation. The presence of larger-amplitude slower dynamics derived from RDC is also in qualitative agreement with the distribution of *B*-factors from the X-ray study (Figure 1a).

Measurement of RDCs in five different alignment media is still a substantial undertaking for many experimental systems. We have therefore applied the method using the two data sets that are most independent as judged by the normalized scalar product of the Saupe matrix elements for the two tensors.[12] This results in motional amplitudes that closely reproduce those derived from the analysis using five independent data sets (Figure 1) and results in the rejection of only three additional sites. If we then fit the remaining data (the three least independent tensors), we again extract very similar motional amplitudes (Figure S1, Supporting Information), demonstrating both cross-validation and robustness, even in the presence of few alignment media.

A similar analysis has been applied to N−^{N}H RDC data from lysozyme measured in two different alignment media.[13] No C′−$^{\alpha}$C dipolar couplings were available in this case, and thus an approach presented recently to estimate the non-dynamically averaged alignment tensor from N−^{N}H RDC was used to determine these parameters.[6] In comparison to temperature factors extracted from the 0.9-Å resolution structure[14] (Figure 2a), some correlation is again found, although local motions are also observed in a loop region presenting low *B*-factors (around residue 36). The total χ^2 for the presented residues using the GAF model is 46, while for the same residues the static model gives 1125.

The dependence of this approach on a structural model of the protein requires an assessment of possible aliasing of coordinate error into apparent dynamics. The method is, in practice, quite robust in this respect because anisotropic motion results in coherent averaging with respect to the different alignment tensors that can then be extracted using the GAF approach, while this is not generally the case for structural noise. To illustrate this we have repeated the analysis using lower-resolution lysozyme structures. Dynamic amplitudes extracted from structures with 1.5-[15a] and 2.1-Å[15b] resolution are comparable to those from the 0.9-Å structure (Figure 2b) and demonstrate the insensitivity of the extracted amplitudes with respect to structural noise, at least within this resolution range. Comparison of fits from all sites, irrespective of statistical quality (Figure 2c), illustrates the effects of filtering the parameter fits using the selection criteria.[10]

In conclusion we present a straightforward method for interpreting dynamically averaged RDCs in proteins. We assume a single, effective geometric model and interpret motional averaging in terms of the amplitude of this motion. While this is a simplification of potentially complex motions, the method reduces the number of independent measurements required for the characterization of local motions, and by applying appropriate error analysis we are able to extract apparently robust motional parameters. We note that the method provides an absolute measure of dynamic amplitudes. By comparing these amplitudes to rapid motional parameters derived from spin relaxation, a range of motional regimes can be distinguished in protein G. While the α-helix retains a degree of structural integrity similar to that observed on the faster time scale, different loop regions exhibit large amplitude motions on either fast or slower time scales. We are exploring further experimental systems using this method and are optimistic that this approach will contribute significantly to characterizing slower conformational dynamics in proteins.

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Supporting Information Available: Plot of motional amplitudes extracted from protein G using data from the three least independent alignment media. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Palmer, A. G. *Curr. Opin. Struct. Biol.* **1997**, *7*, 732−737; Kay, L. E. *Nat. Struct. Biol.* **1998**, *5*, 513−517.
(2) Loria, J.; Rance, M.; Palmer, A. G. *J. Am. Chem. Soc.* **1998**, *121*, 2331−2332.
(3) Tolman, J.; Flanagan, J. M.; Kennedy, M.; Prestegard, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9279−9283; Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111−1114; Tolman, J. *Curr. Opin. Struct. Biol.* **2001**, *11*, 532−539.
(4) Meiler, J.; Prompers, J. J.; Peti, W.; Griesinger, C.; Brüschweiler, R. *J. Am. Chem. Soc.* **2001**, *123*, 6098−6107; Peti, W.; Meiler, J.; Brüschweiler, R.; Griesinger, C. *J. Am. Chem. Soc.* **2002**, *124*, 5822−5833; Tolman, J. R. *J. Am. Chem. Soc.* **2002**, *124*, 12020−12030; Briggman, K. B.; Tolman, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 10164−10165.
(5) (a) Brüschweiler, R.; Wright, P. E. *J. Am. Chem. Soc.* **1994**, *116*, 8426−8427. (b) Lienin, S. F.; Bremi, T.; Brutscher, B.; Brüschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 9870−9879. Fischer, M. W. F.; Zeng, L.; Pang, Y.; Hu, W.; Majumdar, A.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1997**, *119*, 12629−12642.
(6) Bernadó, P.; Blackledge, M. *J. Am. Chem. Soc.* **2004**, *126*, 4907−4920.
(7) GAF-averaged RDCs are calculated using eq 6 in ref 6.
(8) Ulmer, T. S.; Ramirez, B. E.; Delaglio, F.; Bax, A. *J. Am. Chem. Soc.* **2003**, *125*, 9179−9191.
(9) Derrick, J. P.; Wigley, D. B. *J. Mol. Biol.* **1994**, *243*, 906−918.
(10) To accept a fit the local χ^2_i must be $<\chi^2_{thresh}$ and error function width ($\Delta(\sigma_i) < \Delta_{thresh}(\sigma)$. Appropriate χ^2_{thresh} values for the available degrees of freedom were determined for each fit assuming 95% confidence limits. $\Delta_{thresh}(\sigma)$ was determined empirically. The fit is excluded if either of these tests failed unless a reduction in χ^2 is observed that is statistically significant ($p < 0.05$).
(11) Hall, J. B.; Fushman, D. *J. Biomol. NMR* **2003**, *27*, 261−275.
(12) Sass, J.; Cordier, F.; Hoffman, A.; Rogowski, M.; Cousin, A.; Omichinski, J.; Löwen, H.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 2047−2055.
(13) Schwalbe, H.; Grimshaw, S.; Spencer, A.; Buck, M.; Boyd, J.; Dobson, C.; Redfield, C.; Smith, L. J. *Protein Sci.* **2001**, *10*, 677−688.
(14) Walsh, M. A.; Schneider, T. R.; Sieker, L. C.; Dauter, Z.; Lamzin, V. S.; Wilson, K. S. *Acta Crystallogr., Sect. D* **1998**, *54*, 522−546.
(15) (a) Artymiuk, P. J.; Blake, C. C. F.; Rice, D. W.; Wilson, K. W. *Acta Crystallogr., Sect. B* **1982**, *38*, 778−788. (b) Bradbrook, S.; Deacon, A.; Habash, J.; Helliwell, J. R.; Helliwell, M.; Nieh, Y. P.; Snell, E. H.; Trapani, G.; Thompson, A. W.; Campbell, J. W.; Allinson, N. M.; Moon, K.; Ursby, T.; Wulff, M. *Proc. SPIE - Int. Soc. Opt. Eng.* **1995**, *2521*, 160.

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