Identification of slow correlated motions in proteins using residual dipolar and hydrogen-bond scalar couplings

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Despite their importance for biological activity, slower molecular motions beyond the nanosecond range remain poorly understood. We have assembled an unprecedented set of experimental NMR data, comprising up to 27 residual dipolar couplings per amino acid, to define the nature and amplitude of backbone motion in protein G using the Gaussian axial fluctuation model in three dimensions. Slower motions occur in the loops, and in the β-sheet, and are absent in other regions of the molecule, including the α-helix. In the β-sheet an alternating pattern of dynamics along the peptide sequence is found to form a long-range network of slow motion in the form of a standing wave extending across the β-sheet, resulting in maximal conformational sampling at the interaction site. The alternating nodes along the sequence match the alternation of strongly hydrophobic side chains buried in the protein core. Confirmation of the motion is provided through extensive cross-validation and by independent hydrogen-bond scalar coupling analysis that shows this motion to be correlated. These observations strongly suggest that dynamical information can be transmitted across hydrogen bonds and have important implications for understanding collective motions and long-range information transfer in proteins.

protein dynamics | slow motions | correlated

Molecular dynamics, manifest in backbone and side-chain mobilities, play a crucial role in protein stability and function (1–4). The accurate characterization and understanding of protein motions thus adds an additional dimension to the structural information derived from genomics projects (5, 6). Although local backbone fluctuations on the picosecond to nanosecond time scale have been the subject of detailed characterization using NMR (7, 8) and molecular dynamics simulations (2), slower motions, in the submicrosecond to second range, remain poorly understood. Relaxation dispersion has been used to successfully identify sites of conformational exchange between states experiencing different chemical shifts in peptides (9) and proteins (10), but specific geometric motional models are often difficult to extract from these data. Slow time scales are, however, of particular interest because functionally important biological processes, including enzyme catalysis (11), signal transduction (12), ligand binding, and allosteric regulation (13), as well as collective motions involving groups of atoms or whole amino acids (14), are expected to occur in this time range. Residual dipolar couplings (RDCs) report on averages over a very broad time scale (15, 16). Recent studies have exploited the orientational averaging properties of RDCs to characterize the amplitude and direction of motions of NH vectors (17–19) or to study local variations in position and dynamics of the amide proton (20, 21). Despite this activity, key questions remain concerning the nature and amplitude of slower backbone motions on the peptide chain (22), whose resolution is not only fundamental to our understanding of protein function but also important for the accurate incorporation of conformational disorder into protein structure elucidation (23).

In this work, we have assembled an extensive data set, comprising up to 27 dipolar coupling interactions that sample 6 different directions throughout the peptide plane, to define the nature and amplitude of protein backbone motion in an IgG domain of streptococcal protein G (henceforth called protein G). A detailed study of fast motion along the backbone of 10 point-mutants of this protein revealed covariation of dynamic parameters compared with the wild-type protein (24). Here, we directly investigate the presence of correlated motion using a combined analysis of RDCs and scalar couplings across hydrogen bonds. This analysis reveals a striking distribution of dynamics along the protein backbone and identifies a long-range network of motions involving dynamic correlations between amino acids connected via hydrogen bonds. Because of the large amount of experimental data, the results are very robust as validated by a systematic jack-knife procedure. Potential aliasing because of imprecision of the structural model is also shown to be negligible. Independent confirmation of the presence of this dynamic network is provided through hydrogen-bond scalar couplings (HBCs). Agreement between predicted and experimental scalar couplings is substantially improved by using the RDC-derived dynamic distributions of hydrogen-bond geometries. The results suggest that long-range dynamical information is transferred across an entire four-strand β-sheet in protein G and that this motion is transmitted via the interstrand hydrogen bonds. These findings have clear implications for our understanding of collective motions and information transfer in proteins.

Materials and Methods

Experimental Methods. In addition to data from ref. 20 containing five sets of $\text{D}_{\text{HHN}}$, $\text{D}_{\text{DCN}}$, and $\text{D}_{\text{DC-C}}$, couplings, two sets of $\text{D}_{\text{HN}}$, $\text{D}_{\text{HC}}$, $\text{D}_{\text{HC(C-o)}}$, $\text{D}_{\text{CN}}$, and $\text{D}_{\text{CC}}$ RDCs were measured in a perdeuterated sample, aligned in bacteriophage (25) and lamellar phases (26). Spectroscopic parameters and conditions were applied identically as described in ref. 27.

Calculation of RDCs Averaged Under 3D Gaussian Axial Fluctuation (GAF) Motion. The 3D GAF amplitudes were determined by minimizing the function

$$\chi^2(\sigma_\alpha, \sigma_\beta, \sigma_\gamma) = \sum_m \sum_i \left( \frac{D^{\exp}_{m,i} - (D^{\text{3D-GAF}}_{m,i})}{\Delta_{m,i}} \right)^2,$$

where $D^{\exp}_{m,i}$ is the experimental RDC, $D^{\text{3D-GAF}}_{m,i}$ is the calculated RDC from the 3D GAF model, and $\Delta_{m,i}$ is the error in the experimental determination. The parameters $\sigma_\alpha$, $\sigma_\beta$, and $\sigma_\gamma$ are the Gaussian axial fluctuations along the α, β, and γ directions, respectively. The 3D GAF motion was chosen because it provides a good model for the slow motions observed in the protein.

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Abbreviations: GAF, Gaussian axial fluctuation; HBC, hydrogen-bond scalar coupling; RDC, residual dipolar coupling.

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where $\langle D_{i,m} \rangle^{3DGAF}$ is calculated from the structural coordinates, described by the 1.1-A crystal structure (Protein Data Bank ID code 1igd) (28) with amide protons added, and $\Delta_{i,m}$ is a data-set-specific weighting factor (see below). $\langle D_{i,m} \rangle^{3DGAF}$ is defined in terms of $\sigma_\alpha$, $\sigma_\beta$, and $\sigma_\gamma$, the amplitude of motions (in degrees) about the axes shown in Fig. 1.

After alignment tensor determination (see below), the coordinates of the protein were rotated into the principal axis system of the alignment tensor, and the averaged coupling was calculated as a function of $\sigma_\alpha$, $\sigma_\beta$, and $\sigma_\gamma$, the amplitude of reorientational motions about the three axes using (16, 17)

$$\frac{(D)_{3DGAF}}{D_{max}} = \sqrt{\frac{3\pi}{5}} \left\{ (Y_{20})_{3DGAF} + \frac{3}{8} R((Y_{22})_{3DGAF} + (Y_{2-2})_{3DGAF}) \right\},$$

where

$$\langle Y_{2M} \rangle_{3DGAF} = \sum_{i',j'} e^{-i\phi i'j'} \sum_{n,n'} e^{-i\phi n} \sum_{m,m'} e^{-i\phi m} \sum_{i,j} d_{i,j}(-\theta_i)d_{i',j'}(\theta_{i'})$$

and

$$\langle D_{i,m} \rangle^{3DGAF} = 2 \sum_{i',j'} \sum_{n,n'} \sum_{m,m'} e^{-i\phi j} e^{-i\phi n} \sum_{n',n''} \sum_{m',m''} e^{-i\phi m} \sum_{i,j} d_{i,j}(-\theta_i)d_{i',j'}(\theta_{i'})$$

Further details of the 3D GAF model can be found in refs. 29 and 30.

**Data-Fitting Procedure.** The weighting of the different RDC data sets in Eq. 1 is a key point of our analysis. The fitting procedure designed to extract the three motional amplitudes was performed as follows:

1. Determination of the relative weighting of the 27 different data sets. To avoid overfitting of a particular data type ($D_{i,m}$), we have weighted each of the 27 experimental data sets to equalize the total target function of each data set when fit to the static structure, which essentially means scaling the denominator $\Delta_{i,m}$ for each of the coupling types from each medium so that $\kappa^2$ is the same when fit to the static structure. This method has the advantage of not favoring any motional mode (by affecting one coupling type more than the others) from the beginning of the procedure.

2. Determination of the five parameters describing each of the seven molecular alignment tensors was initially performed by using all 1D couplings, in comparison with the static structure using the program module (31). This method again does not favor any motional mode from the beginning of the procedure.

3. Fitting of $\sigma_\alpha$, $\sigma_\beta$, and $\sigma_\gamma$ motional amplitudes, using Eqs. 1-3.

4. Step 2 is repeated but this time using the RDCs from vectors that have been identified in step 3 as having the lowest amplitude motions.

5. The final amplitudes of $\sigma_\alpha$, $\sigma_\beta$, and $\sigma_\gamma$ (those presented in this work) are now calculated by repeating step 3 with respect to these nondynamically averaged, and therefore more precise, alignment tensors.

**Cross-Validation of the 3D GAF Dynamic Description.** The entire procedure was repeated with complete data sets removed from the analysis to cross-validate the accuracy of the local dynamic amplitudes. Experimental RDCs that were not used in the analysis were compared with values calculated using the dynamic parameters derived from the retained experimental data.

Three structural models were used, the x-ray crystallographic structures with Protein Data Bank ID codes 1igd (1.1-A resolution), 1pgb (1.9-A resolution), and the RDC-refined NMR structure 1pc7 (20).

**Error Analysis.** Uncertainty in the values of $\sigma_\alpha$, $\sigma_\beta$, and $\sigma_\gamma$ was estimated by using noise-based Monte Carlo sampling procedures from random sampling of the simulated distributions of each coupling. The width of the distributions is based on the relative weightings per RDC described above, and an overall scaling applied to the entire data set that is calibrated such that the average value over all sites of the total reduced $\chi^2$ determined from all couplings is approximately equal to the $P = 0.05$ expected value. The uncertainty in the dynamic amplitudes is therefore appropriate if the model is correct.

**Calculation of HBCs.** Scalar coupling across hydrogen bonds were calculated by using the following equation taken explicitly from Barfield (32):

$$J_{NC}^{\beta}(\theta_2, \rho, r_{HO}) = \{ -1.31 \cos^2 \theta_2 + [0.62 \cos^2 \rho + 0.92 \cos \rho + 0.14 \sin^2 \theta_2] \times \exp[-3.2(r_{HO} - r_{HO}^0)] \} \text{Hz},$$

where $r_{HO}$, $\theta_2$, and $\rho$ are the H–O’ distance, the HO’C’ angle and the H–O’C’–N dihedral angle, respectively.

Three models were used to incorporate the observed motion into the calculation of scalar coupling across hydrogen bonds: (1) uncorrelated motion, (2) correlated motion, and (3) anticorrelated motion.
1. Noncorrelated motion was incorporated by randomly selecting three rotation amplitudes from Gaussian distributions of width given by the experimentally determined $\sigma_a$, $\sigma_b$, and $\sigma_c$ parameters for each plane and by applying these rotations sequentially to the mean plane orientation taken from the crystal structure. Assuming plane $i$ is hydrogen bonded to plane $j$, this procedure is applied to both planes, and for each of 10,000 random combinations, the parameters $s_{ijAC}$, $\theta_2$, and $p$, and consequently $\gamma_{ij}$ are calculated for the $ij$ pair.

2. Correlated motion was simulated by using an identical procedure except that the selection of rotations for partner $i$ are correlated with those of partner $j$. When one peptide plane partner samples a conformation that is tilted away from the equilibrium position, the second partner also moves in the same direction. This procedure was used to predict couplings for each of 10,000 pairs of conformers, which then were averaged linearly.

3. Anticorrelated motion was simulated by using an identical procedure except that the selection of rotations for partner $i$ are inversely correlated with those of partner $j$. When one peptide plane partner samples a conformation that is tilted away from the equilibrium position, the second partner moves in the opposite direction. This procedure was used to predict couplings for each of 10,000 pairs of conformers, which were then averaged linearly.

**Results**

**Determination of Long Time-Scale Dynamic Modes and Amplitudes from RDCs.** We have recently demonstrated (33, 34) the utility of applying a simple physical model of peptide reorientation [1D GAF (35)] to study conformational averaging by combining $^{1}D_{HN}$ RDCs from more than one alignment medium. Although this approach has been shown to be relatively insensitive to structural noise (36), the model nevertheless assumes that motion about the $C_{\alpha}^-\rightarrow C_{\alpha}^+$ axis connecting sequential amino acids is dominant and that reorientations about orthogonal axes are less ample and therefore cannot provide further insight into the presence of more complex reorientational modes. Motional anisotropy can be fully addressed by using the 3D GAF model of peptide plane dynamics (29, 30) which was originally developed for the interpretation of spin-relaxation data. The 3D-GAF model allows three independent modes, about each of the three orthogonal axes shown in Fig. 1a. This model is used here to analyze the backbone dynamics of protein G from RDCs measured in multiple alignment media.

**Distribution of Motion Along the Protein G Backbone.** The 3D GAF motion is defined in terms of $\sigma_a$, $\sigma_b$, and $\sigma_c$ along each individual peptide plane. This data set comprises published $^{1}D_{HN}$, $^{1}D_{CC}$, and $^{1}D_{CC}$ couplings measured in five different alignment media (20) and data sets from two alignment media measured in one of our laboratories, comprising $^{1}D_{HN}$, $^{1}D_{CH}$, $^{1}D_{CH}$, $^{1}D_{HC}(\alpha-1)$, $^{1}D_{HN}$, and $^{1}D_{CC}$ couplings (27) (Fig. 1b).

The distributions of $\sigma_a$, $\sigma_b$, and $\sigma_c$ along the protein backbone are shown in Fig. 2 (uncertainties in $\sigma_a$, $\sigma_b$, and $\sigma_c$ were estimated to be of the order of $5^\circ$, $4^\circ$, and $5^\circ$, respectively; see Materials and Methods). In general, reorientation about the $C_{\alpha}^-\rightarrow C_{\alpha}^+$ provides the dominant dynamic mode along the peptide chain, exhibiting larger amplitude motions than those occurring around the orthogonal $\alpha$ and $\beta$ axes, respectively, in the peptide plane and orthogonal to the plane. In all secondary structural elements except $\beta$-strand $\beta_3$, the $\sigma_a$ axis is effectively undetectable, whereas in the $\alpha$-helix the $\gamma$-motion has a nearly uniform amplitude of $12 \pm 3^\circ$. A striking pattern is immediately perceptible in the $\beta$-sheet, where alternating large- and small-amplitude motions about the $\gamma$ axis can be observed in the hydrogen-bonded strands $\beta_1$, $\beta_2$, and $\beta_4$. These motions are associated with smaller-amplitude $\beta$-motions that are regularly interspersed in the intervening peptide planes that show negligible $\gamma$-motion (Figs. 2 and 3). Notably these motional modes appear to be coupled across the sheet, following chains of hydrogen-bonded peptides that experience similar amplitude motions and align orthogonal to the direction of the peptide backbone. This pattern is depicted in Fig. 4, where the amplitudes of $\gamma$-motions are represented as conformational ensembles at each peptide plane. We note that strand $\beta_2$, immediately after the flexible 14–17 loop and presenting the most solvent-exposed surface, exhibits high-amplitude dynamics along the strand that successively diminish in amplitude from residue 19 to residue 24.

**Cross-Validation of the 3D GAF Dynamic Description.** Because of the large number of RDCs available, we are able to apply cross-validation techniques to establish the accuracy of the extracted dynamics. (For details, see Supporting Text, Tables 1–8, and Figs. 8–15, which are published as supporting information on the PNAS website.) The analysis was thus repeated seven times after removing the $^{1}D_{HN}$ data from each individual alignment medium in turn. Experimental values for the removed couplings were then compared with those predicted by using the dynamic and static models. In all cases, significant overall improvement is found compared to the static model. The total $\chi^2$ over all seven simulations is a factor of 2 smaller for the dynamic model. As expected if the motional models are valid, the static model tends to
to overestimate the predicted couplings (Fig. 10). This procedure was repeated, this time by removing all couplings from each of the seven alignment media. Again, a systematic site-by-site improvement is also found by using the dynamic model (Figs. 11–13). Notably, couplings from the most independent data set (aligned in negatively charged polyacrylamide gel) also are found to be significantly better predicted using the motional models determined from the remaining data (Fig. 14). Together, these results provide strong support for the proposed dynamic model determined in this work.

Sensitivity to Structural Imprecision. We have tested the sensitivity of our approach to uncertainties in the atomic coordinates used to model the average conformation, using a structure refined using 1DC/H11032 N and 1DC/H9251 C/H11032 RDC from five alignment media, resulting in essentially indistinguishable motional amplitudes to those shown in Figs. 2 and 3 (Fig. 8). Cross-validation calculations also show significant improvement with respect to the static analysis by using this model (Fig. 13). Additional analysis with a lower-resolution structure of protein G also gives very similar results (Fig. 9), further demonstrating the robustness of the approach to this source of error, resulting from the large volume of data used to define the local mobility.

Dynamic Averaging of HBCs. The observation of shared dynamic modes extending through interstrand connectivity in the β-sheet is intriguing, particularly in view of the amplitude of the γ-motions in the β-strands, which reach up to $\sigma_\gamma = 23^\circ$. These motions have been investigated further by incorporating the observed dynamic amplitudes into prediction of the strength of trans-HBCs. HBCs represent time averages over a time-scale range similar to the one probed by RDCs (37). We have used an analytical description of $^3J_{NC}$ scalar coupling that was recently parameterized from density functional theory calculations (32) to predict couplings for each of 10,000 conformers derived from Monte Carlo sampling of the 3D GAF distributions of peptide planes containing the two hydrogen-bonding partners (see Materials and Methods). Averages were taken over this ensemble for the 29 hydrogen bonds for which $^3J_{NC}$ scalar couplings have been measured previously (38) (excluding the most flexible loop region 14–17). Experimental values are significantly better reproduced by using this dynamic model than by using the static model (Fig. 5). This finding applies for HBCs throughout the molecule and for HBCs of both secondary structural elements, further validating the presence of the proposed motional modes in this protein.

Motions Across the β-Sheet Are Correlated. The 3D GAF amplitude pattern across the β-sheets (Fig. 4) raises the question of whether these motions are mutually correlated. However, correlated motions between peptide planes cannot be identified by using local RDCs alone. This question can be addressed by including spin–spin couplings between the peptide planes, which are available in form of trans-HBCs.

We therefore have tested a simple model of correlated motion that assumes that when the peptide plane providing the hydrogen-bond donor samples a conformation that is tilted away from the equilibrium position, the acceptor plane moves in the same direction.
milliseconds time scale (relevant for the NH bond reorientational averaging up to the nanosecond time-scale window relevant for RDCs, the dynamic backbone motions as they propagate across the protein core (Y8, L10, I12, F57, and V59) (Fig. 6). These hydrophobic side chains that participate in the stabilization of the observed dynamic modes and the presence of strongly extensive interactions between buried hydrophobic side chains. The high stability of protein G is thought to be linked to the particular features that may provide the key to these motions. Because of the broad time-scale window relevant for RDCs, the dynamic information observed here is complementary to motional amplitudes derived from spin-relaxation measurements. The 3D GAF description allows determination of generalized order parameters relevant for the NH bond reorientational averaging up to the millisecond time scale ($S_{\text{slow}}^2$), which can be compared with order parameters extracted from $^{15}$N relaxation that reflect on fast reorientation (picosecond to nanosecond) of the same internuclear vector ($S_{\text{fast}}^2$) (ref. 39; see Fig. 7). Overall, the profiles are comparable: $S_{\text{fast}}^2$ values in residues 22–48, comprising the $\alpha$-helix and the $\beta$2- and $\beta$3-helix loops, are very similar, both in terms of absolute value and local differences. Certain sites, in particular the NH vector of Gly-46, have similarly high-amplitude motion over both fast and slow time scales, indicating that the main component of this motion is occurring over the picosecond to nanosecond range and that negligible additional motions are occurring on the slower time scales. It should be noted that no additional scaling has been applied to match to $S_{\text{fast}}^2$ values from relaxation. Indeed, the principal factor affecting the scale of the extracted amplitudes using this technique is the choice of RDCs used for tensor determination. In our approach, an initial analysis of the data identifies the vectors with least motion, and these vectors then are used in a second, final analysis, to determine tensors as precisely as possible, with the least potential influence of dynamic averaging (see Materials and Methods).

Significant differences between fast and slow dynamic averaging, however, can immediately be recognized. In particular,
the loop region between residues 14 and 17, previously identified as a melting hot spot in protein G (40), and the following 4 residues of strand $\beta_3$, exhibit lower-order parameters, corresponding to additional motion that must be occurring on the slower time scale. If we examine the order parameters of sites involved in the previously discussed correlated motions across the $\beta$-sheet, we find that the $\Omega_{\text{tan}}$ values of the sites exhibiting larger $\gamma$-motions (K9, V11, T58 and T60) are systematically lower (0.68 ± 0.03) than the respective fast motion $\Omega_{\text{tan}}$ for these sites (0.86 ± 0.01), indicating that the correlated motions traversing the $\beta$-sheet are indeed occurring on the slower time range extending from tens of nanoseconds to a few milliseconds.

A conceptually very different approach to interpretation of RDCs, using molecular dynamics-based ensemble-averaging, identified order parameters in protein G with a similar profile to those shown in Fig. 7 (41), although the amplitude of the motions was slightly smaller.

**Discussion**

In summary, an extensive set of RDCs, in combination with a motional model providing for anisotropic peptide plane reorientation in three orthogonal dimensions, has been used to identify and cross-validate the presence of conformational dynamics in protein G. Comparison with the amplitude and position of rapid motions in the same protein reveals a heterogeneous distribution of slow dynamics in the nanosecond to millisecond time range. Larger-amplitude slower motions occur in the loops, and in the $\beta$-sheet, but are absent in other regions of the molecule, including the $\alpha$-helix. In the $\beta$-sheet an alternating pattern of dynamics along the peptide sequence is found to form a long-range network across the $\beta$-strands, reminiscent of a standing wave. The alternating nodes match the alternation of strongly hydrophobic side chains buried in the core of the protein that apparently provides the anchor-point for the undulating motion across this sheet. Importantly the extracted motional modes significantly improve the prediction of experimentally measured scalar couplings across hydrogen bonds throughout the molecule. This analysis also strongly suggests that the motion is correlated across, and propagated through interstrand hydrogen bonds. Such clear evidence that dynamical information is transmitted across hydrogen-bond networks carries important implications for understanding the mechanism of information transfer in proteins occurring in processes such as allosteric regulation.

The amplitude of the collective motion increases across the $\beta$-sheet with the highest amplitude in strand $\beta_2$. This finding may be directly relevant for the function of protein G: the residues exhibiting the highest level of flexibility coincide precisely with the sites participating in the interaction of protein G with its physiological partner, the antigen-binding domain of IgG. This interaction is mediated by means of a complete antiparallel intermolecular $\beta$-sheet involving hydrogen bonds at residues 16, 18, and 20. The increased dynamics at these sites are predominantly due to $\gamma$-motion, perpendicular to the strand direction with largest motional excursions for the NH and CO bonds. These modes are precisely those that offer the maximum structural sampling necessary for the hydrogen-bonding atoms to successfully locate the partner strand and thereby form the required hydrogen-bonding network. We therefore propose that molecular interaction is facilitated by the increased conformational sampling due to this collective motion.

In this work, we have obtained evidence from RDCs and HBCs that dynamical information can be transmitted in the form of slow collective modes across hydrogen-bond networks. Although this kind of transmission of dynamics has been proposed (42), it has never been experimentally observed, and because of the long time scales involved is hard to access by current molecular dynamics simulations.

The existence of these slow motional modes extending across the entire $\beta$-sheet carries clear implications for understanding the mechanisms of long-range signal propagation in proteins. In the case of the protein G, these findings illustrate how the protein harnesses thermal motions through specific dynamic networks to enable molecular function at the interaction site.

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