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Dipolar Couplings in Multiple Alignments Suggest α Helical Motion in Ubiquitin

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The structural dynamic characterization of biomolecules in solution can contribute to the understanding of key biochemical processes such as protein folding, transport, and catalysis.[1] Through the last years residual dipolar couplings[2,3] (RDCs) have been proven to be powerful tools in protein structure elucidation. Recently, analyses were suggested that characterize dynamics utilizing RDCs.[4−8] RDCs of backbone N−H^N vectors measured in 11 different alignment media were analyzed with respect to structure and dynamics in a model-free way in terms of generalized order parameters and motional anisotropies[4,5] under the assumption that the structure does not change because of the alignment media.[9] The anisotropies in the central α-helix were found to be strikingly uniformly distributed. In this communication, these parameters are further interpreted in terms of physically feasible cooperative reorientational motion of the helix with respect to the core of the protein. Residual dipolar couplings of N−H^N vectors collected in at least five different alignment media allow the reconstruction of the following five parameters per N−H^N vector: average position of each internuclear vector θ_{eff},ϕ_{eff} (identical to $\theta^1_{eff},\phi^1_{eff}$ in lit.[5]), an order parameter S^2_{rdc} that reflects the amount of order of the orientational distribution and that by contrast to the regular relaxation-derived Lipari Szabo order parameter S^2_{LS} covers all time scales up to approximately 10 ms. In addition, the anisotropy of the orientational distribution was characterized by an amplitude η_{rdc} which represents the ellipticity of the distribution and an angle ϕ_{rdc} (identical to $\bar{\phi}_{rdc}$ in lit.[5]) which measures the angle of the long axis of the ellipse with a molecule fixed reference orientation.

A simple model distribution (diffusion in a cone) that can be fitted to the five model-free parameters ($\theta_{eff},\phi_{eff},S^2_{rdc},\eta_{rdc},\phi_{rdc}$) puts the N−H^N vectors on an elliptical distribution with its center pointing along the effective N−H^N direction (θ_{eff},ϕ_{eff}), with the principal asymmetry axis specified by ϕ_{rdc}, and the principal axes *a* and *b*. For the α-helix in Ubiquitin the five parameters were determined for the N−H^N vectors of six amino acids: Ile23, Asn25, Lys27, Lys29, Ile30, and Asp33. The effective orientations of the N−H^N are very similar as is expected for N−H^N vectors in an α-helix (Table 1). Less obvious, the angles ϕ_{rdc} that define the principal directions of the anisotropy are also strikingly similar for helical N−H^N vectors, taking an average value of −191 ± 14° (compared to −180 ± 69° for all amino acids; for the significance of these findings see Supporting Information, a). Hence, the variation of ϕ_{rdc} in the helix is notably small and may be indicative of correlated reorientational behavior of the N−H^N vectors.

In Table 1 we report the extreme values (min/max) of the ranges of motional parameters that are consistent with our experimental data and uncertainties of ref 5. For example, *a*,*b* range between 9°,0° and 19°,15°. (For the fitting procedure and a detailed discussion see Supporting Information, b). These parameters take similar values for five of the six residues exhibiting significant amounts of asymmetry. Only for Lys27 are similar values of *a* and *b* found. The motion along the small axis of the ellipse is clearly increased compared to that for the other amino acids in the α-helix. To a lower extent this is also true for Asn25 and Asp33; however, a significant difference between *a* and *b* remains in all of the other five cases (Figure 1).

Table 1. Parameters Obtained for the α-Helix of Ubiquitin (min/max[a])

i	θ^i_{eff} [deg]	ϕ^i_{eff} [deg]	ϕ^i_{rdc} [deg]	$\langle Y^i_{20}\rangle$ [1]	$\langle Y^i_{22}\rangle$ [1]	S^i_{rdc} [1]	η^i_{rdc} [1]	a^i [deg]	b^i [deg][b]
23	85	22	−205	0.57/0.63	0.07/0.13	0.92/1.00	0.15/0.30	16/21	0*/0*
25	95	−9	−177	0.53/0.63	0.01/0.05	0.84/1.00	0.02/0.13	9/19	0*/15
27	101	20	−205	0.50/0.56	0.01/0.04	0.79/0.89	0.02/0.11	21/21	0*/19
29	88	−5	−190	0.53/0.62	0.03/0.09	0.84/1.00	0.07/0.24	15/23	0*/ 8
30	94	11	−198	0.55/0.63	0.03/0.11	0.87/1.00	0.07/0.26	15/22	0*/ 4
33	84	−5	−169	0.53/0.63	0.01/0.06	0.84/1.00	0.01/0.15	11/18	0*/16
aver	91	6	−191	0.54/0.62	0.02/0.08	0.85/0.99	0.05/0.20	16/21	0*/12

[a] Left and right values reflect minimum and maximum. [b] The * signifies that a *b* value smaller than 0 is found.

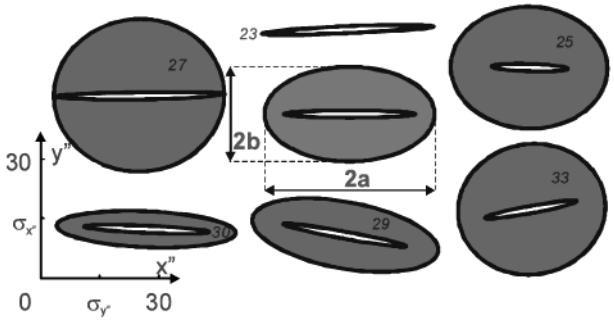

Figure 1. Elliptic traces for the six amino acids and the average (black filling) in the coordinate system C″ (figure S1) where the average effective orientation of all N−H^N vectors is parallel to the *z*″-axis, and the average major component of the dynamical distribution is along the *x*″-axis. The smaller (larger) ellipse corresponds to the left (right) values in Table 1.

Since all vectors show very similar structural and motional parameters, we investigate whether a simultaneous motion of the whole helix can be accommodated to explain a part of the obtained individual motional parameters. It should be stressed that this cooperativity cannot be inferred directly from the N−H^N dipolar couplings. It is however possible to study the implications of such behavior both with respect to homonuclear NOE data and molecular mechanics energies. Assuming such cooperative behavior, the average motional distribution can be determined by averaging the five time-averaged spherical harmonics $\langle Y^i_{2M}(\theta,\phi)\rangle$ over the six residues. To avoid an artificial reduction

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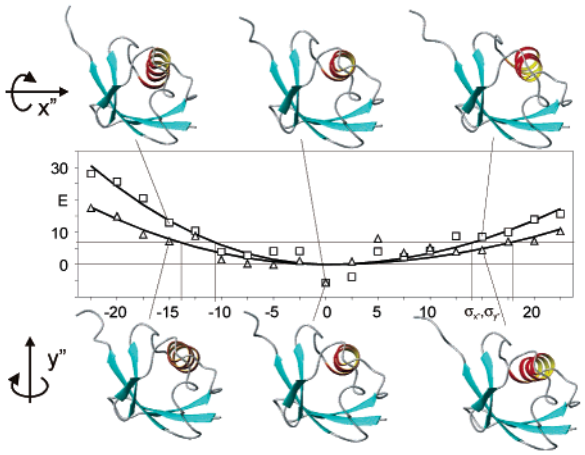

Figure 2. Results of the computational analysis: The figure shows ubiquitin in the coordinate frame C″ with the helix rotated by −15°, 0°, and +15° about the *y*″-axis (top row/open square) and the same structure with the helix rotated about the *x*″-axis (bottom row/open triangle). The central diagram summarizes the relative energies of the resulting conformations.

of S due to averaging, the N sets of spherical harmonics were rotated into a common coordinate system prior to averaging by applying $R(-\phi^i_{\text{eff}}, -\theta^i_{\text{eff}}, 0)$. For the N parallel N−H^N vectors in this coordinate system the spherical harmonics $\langle Y^i_{2M}(\theta,\phi)\rangle$ (with $1 \le i \le N$) obtained should be consequently similar and therefore also the parameters $\bar{\theta}^i_{\text{eff}}, \bar{\phi}^i_{\text{eff}}, \phi^i_{\text{rdc}}$ and a^i, b^i. In this special case average parameters $\bar{\theta}_{\text{eff}}, \bar{\phi}_{\text{eff}}, \phi_{\text{rdc}}$ and $\bar{a}$, $\bar{b}$ valid for the cooperative part of the motion are obtained by analyzing the averages $(1/N)$ $\sum_{i=1}^{N}\langle Y^i_{2M}(\theta,\phi)\rangle$. Further we introduce a coordinate system C″ which is rotated by $R(-\phi_{\text{eff}}, -\theta_{\text{eff}}, -\phi_{\text{rdc}})$ with respect to the reference coordinate system of the molecule C (Supporting Information, c). In this coordinate system the axis of the helix is parallel to z'', $\bar{a}$ is parallel to x'', and $\bar{b}$ is parallel to y''.

In Figure 1 a 3D backbone structure of ubiquitin is shown together with the elliptic distributions of all six N−H^N vectors. The averaged values are $\bar{a} = 21°$ and $\bar{b} = 12°$, indicating that the reorientational motion of the helix is larger toward the indentation formed by the β-sheet than toward the tips of the β-sheet. The size of both parameters can be reduced by ∼33% within the error of the experiment; however, the asymmetry is the minimal that is consistent with the data.

To investigate whether this model implying collective helix motion is consistent with distances derived from NOESY intensities, the following analysis was performed. An energy-optimized structure of ubiquitin is obtained by an energy minimization[10] in the presence of 2754 NOE distance[11] restraints. Starting from the resulting coordinates the helix was rotated in steps of 2.5° up to a maximum of ±22.5° about the two principal axes of the ellipse, fixing the center of the helix in its relative position toward the β-sheet. For the resulting relative position of the backbone atoms in the α-helix and the β-sheet the position of all atoms in side chains and loop regions was again optimized in the Charmm22 force field[10,11] using the NOE distances as restraints. For the details of the modeling see Supporting Information, d.

In the resulting structures no distance obtained from the NOESY spectra was violated by more than 0.2 Å. In Figure 2 the resulting Charmm22 energies are plotted relative to the reference structure. Many local minima make an analysis of the result difficult, and the incorporation of the NOE restraints prohibits the interpretation of the absolute energies obtained. Therefore, a parabolical potential for each of the rotations about the x'' and y'' axes by positive and negative angles was fitted according to $E = p_{\pm x'',\pm y''} \cdot \sigma^2_{x'',y''} + E_0$ with four individual force constants $p_{\pm x'',\pm y''}$. The higher energy that is necessary for rotations in the direction of the small axes compared to a rotation in the direction of the large one is clearly obtained. Both curves show a similar asymmetry, which reflects a higher energy for rotations with negative angles. For negative angles the α-helix approaches the β-sheet while it departs from it for rotations with positive angles.

According to Figure 2, to achieve a maximal deviation of $a_{\min} = -14°$, $a_{\max} = 18°$, $b_{\min} = -11°$, $b_{\max} = 14°$, a maximal and minimal energy of 6 kcal/mol would be required. This makes such correlated helical excursions feasible from a qualitative point of view. A more quantitative assessment of the total energy changes would, however, require the inclusion of the solvent as well as local relaxation of the modified structures.

In conclusion, we could show that the RDC data of six analyzable N−H^N vectors of the central helix in ubiquitin lead to very similar model-free parameters with significant motional anisotropies. This is compatible with a model in which all N−H^N vectors of the α-helix of ubiquitin exhibit correlated anisotropic excursions with amplitudes of the order of 21° and 12° along the two axes x'' and y'', respectively. Such motion contradicts neither NOE data nor molecular force-field calculations. We are currently pursuing cross-correlated relaxation measurements between distant vectors[12] to investigate the possible cooperativity. Very recently, P. Pelupessy et al.[13] suggested a cooperative motion of subsequent N−H^N vectors in proteins based on cross-correlated relaxation measurements.

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Supporting Information Available: Computation details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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