Protein Structures

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Side-Chain Orientation and Hydrogen-Bonding Imprint Supra-τ_c Motion on the Protein Backbone of Ubiquitin**

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Knowledge about protein dynamics is crucial for the understanding of protein function.[1] NMR spectroscopy can characterize the amplitudes and rates of motions that are either faster than the rotational correlation time τ_c (sub-τ_c motion) with heteronuclear relaxation experiments or between approximately 50 μs and 10 ms (μs/ms motion) with relaxation dispersion.[2,3] The extent of motions occurring in folded proteins on the time scale between the rotational correlation time τ_c and the μs/ms range (supra-τ_c motion) has been a matter of debate.[4] However, the functional relevance of such motions has recently been shown for the aggregation rate of natively unfolded proteins involved in neurodegenerative diseases.[5] Very recently, motions on this supra-τ_c time scale have been observed in a 0.2 μs molecular dynamics simulation of ubiquitin.[6]

Residual dipolar couplings (rdcs) were recognized early on as an ideal tool to widen the time window of dynamics that can be characterized by NMR spectroscopy, since they are sensitive to motional averaging occurring over the sub- *and* supra-τ_c time scales (ps to ms).[4] We recently analyzed NH rdcs of ubiquitin measured in 31 different alignment conditions and derived the order parameters $S^2_{rdc} = \frac{4\pi}{5} \sum_{M=-2}^{2} \langle Y_{2,M}(\theta',\phi') \rangle|^{ms}_0 \langle Y^*_{2,M}(\theta',\phi') \rangle|^{ms}_0$.[4c,7] To differentiate between the sub- and supra-τ_c time scale, these order parameters were compared to Lipari–Szabo order parameters S^2_{LS} that are derived from conventional relaxation time measurements and that are only sensitive to the sub-τ_c time scale.[8,9]

Interestingly, a periodic variation of the S^2_{rdc} value can be observed with a periodicity of two residues in the β strands of ubiquitin (amino acids 2–6, 12–16, 41–45, 66–71), while it is largely absent from the S^2_{LS} and exchange-rate data. Most prominently, the S^2_{rdc} values are larger for residues Gln41, Leu43, and Phe45, and smaller for residues Arg42 and Ile44 in the β strand 41–45 (Figure 1). Correspondingly, the side chains of residues Gln41, Leu43, and Phe45 point towards the hydrophobic core (core residues), whereas the side chains of Arg42 and Ile44 are exposed to solvent (exposed residues). Thus, the alternating pattern of the S^2_{rdc} values seems to correlate with the orientation of the side chains towards the solvent or away from it (Figure 2).

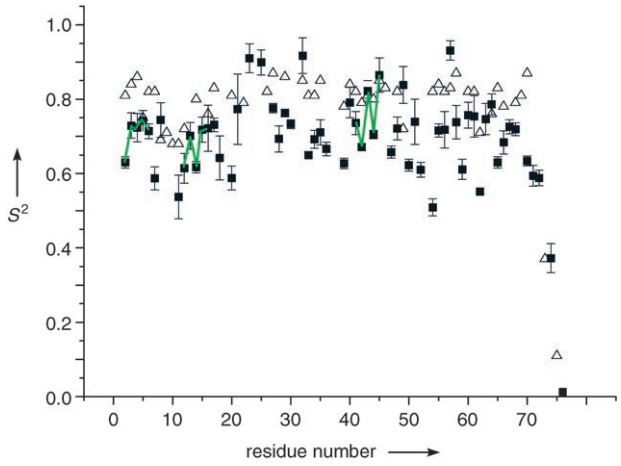

Figure 1. Comparison of the Lipari–Szabo order parameter S^2_{LS} (△) and the rdc derived order parameter S^2_{rdc} (■) as a function of residue number. The marked periodic variations of the S^2_{rdc} values are indicated by lines connecting sequential residues.

The observation that solvent-exposed residues exhibit reduced S^2_{rdc} values relative to core residues holds not only for the β strands but also for the rest of the protein. The amino acids of ubiquitin (1d3z) are color coded in Figure 3 according to the S^2_{rdc} value of the backbone amide groups. Residues with less-mobile NH vectors (blue and green) have side chains predominantly pointing towards the hydrophobic core (Figure 3a), while for those with more-mobile NH vectors (yellow, orange, and red), the side chains are solvent-exposed (Figure 3b).

To investigate this effect quantitatively, an order parameter for only the supra-τ_c time scale is derived: $S^2_{rdc}/S^2_{LS} = \frac{4\pi}{5} \sum_{M=-2}^{2} \langle Y_{2,M}(\theta',\phi') \rangle|^{ms}_{\tau_c} \langle Y^*_{2,M}(\theta',\phi') \rangle|^{ms}_{\tau_c}$. For residues with supra-τ_c motions, we expect a value for S^2_{rdc}/S^2_{LS} smaller than 1, while it should be 1 in the absence of such motions (see the Supporting Information).

Core residues show an average S^2_{rdc}/S^2_{LS} value of 0.91 ± 0.02 as compared to 0.86 ± 0.02 for solvent-exposed residues

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

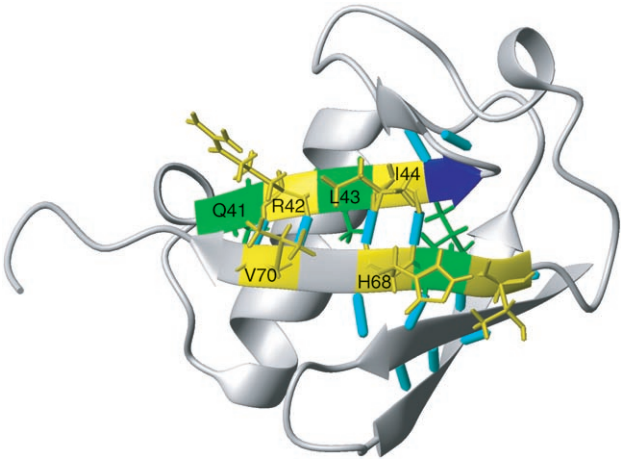

Figure 2. The residues 41–45 and 66–71 of the ubiquitin β strands (1d3z) are color-coded according to the rdc-based order parameters S^2_{rdc} (yellow $0.63 \le S^2_{rdc} < 0.73$, green $0.73 \le S^2_{rdc} < 0.83$, blue $S^2_{rdc} \ge 0.83$, gray unknown). Hydrogen bridges are displayed as cyan rods. The alternating pattern of S^2_{rdc} order parameters in residues 41–45 of the β strand is clearly correlated with the side-chain orientation.

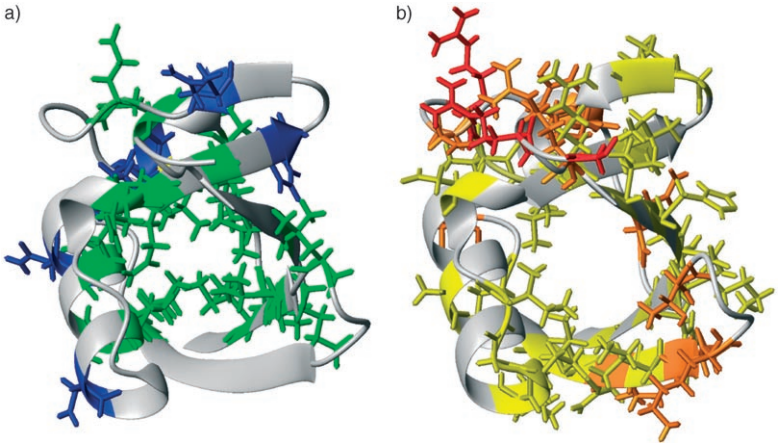

Figure 3. Residues of ubiquitin (1d3z) are color-coded with respect to the rdc-based order parameters S^2_{rdc} (red $S^2_{rdc} < 0.53$, orange $S^2_{rdc} < 0.63$, yellow $S^2_{rdc} < 0.73$, green $S^2_{rdc} < 0.83$, blue $S^2_{rdc} \ge 0.83$, gray unknown) and are distinguished between large (a) and small (b) S^2_{rdc} values. values. Interestingly, most of the residues with larger backbone amide S^2_{rdc} values have side chains pointing to the core (a), whereas residues with solvent-exposed side chains show smaller order parameters (b).

Table 1: Average order parameters for core and exposed residues and for residues involved in 0,1, or 2 hydrogen bonds.[a]

	Exposure				Hydrogen bridges		
	solvent(i)	core(i)	solvent($i-1$)	core($i-1$)	0	1	2
S^2_{LS}	0.80 ± 0.01	0.82 ± 0.01	0.80 ± 0.01	0.81 ± 0.01	0.74 ± 0.02	0.81 ± 0.01	0.83 ± 0.01
S^2_{rdc}	0.69 ± 0.02	0.75 ± 0.02	0.71 ± 0.01	0.70 ± 0.02	0.65 ± 0.03	0.70 ± 0.02	0.77 ± 0.02
S^2_{rdc}/S^2_{LS}	0.86 ± 0.02	0.91 ± 0.02	0.87 ± 0.02	0.86 ± 0.02	0.82 ± 0.05	0.86 ± 0.02	0.90 ± 0.02

[a] The NH_i order parameter was correlated with the side-chain orientation of the same residue (i) and the orientation of the previous residue ($i-1$). Whereas a strong dependence on the side chain orientation is obtained for the first case, no correlation is observed for the second case, thus indicating that mobility is transferred only along the φ and not the ψ angle.

(Table 1), while the exposure of the previous side chain does not have a significant influence (0.86 ± 0.02 versus 0.87 ± 0.02). Furthermore, 90% of the core residues have a S^2_{rdc}/S^2_{LS} value greater than 0.85. In contrast, this is true for only 55% of the solvent-exposed residues (Figure 4a). Thus, the supra-τ_c time order parameter of the NH vector is on average 0.05 ± 0.03 larger if the side chain is buried in the protein core.

We also investigated the influence of backbone hydrogen bonds on the supra-τ_c order parameter S^2_{rdc}/S^2_{LS}. For this, each NH group was classified according to the number of backbone hydrogen bonds on the corresponding peptide plane, including the amino acids NH_i and the preceding carbonyl group CO_{i-1} (see the Supporting Information).

The average S^2_{rdc}/S^2_{LS} values (Table 1) reveal a dependence on the number of hydrogen bonds, and increases from 0.82 ± 0.05 to 0.86 ± 0.02 to 0.90 ± 0.02 as the number of hydrogen bonds increases from zero, to one, to two. Although 83% of residues with two backbone hydrogen bonds have S^2_{rdc}/S^2_{LS} values greater than 0.85, this is true for only 61% of the residues that are involved in only one hydrogen bond (Figure 4b).

In conclusion, side-chain orientation and hydrogen bonds influence supra-τ_c motions of the backbone amide groups to the same extent. The hydrogen-bridge dependency correlates with the secondary structure of the protein whereas solvent exposure is a property of the tertiary structure. It is interesting that the side-chain mobility is apparently transmitted only along the ϕ angle and not along the ψ angle (see the Supporting Information). The investigation of this effect is beyond the scope of this paper. One possibility for this finding could be that the side-chain motion is coupled with pyramidalization of the neighboring NH group in a dissipative manner; this NH group is closer than the NH group of the following amino acid. However, the reason could also be the different rotation barriers around the ϕ and the ψ angles arising from the constitutional difference of these two moieties. The finding that the side-chain orientation influences the supra-τ_c motions of the backbone has been independently observed by Blackledge and co-workers.[10] Alignment tensor fluctuations that have been proposed[13] as an alternative to supra-tc-motion would affect parallel NH vectors identically. However, as NH vectors are parallel in β-sheets, such fluctuations do not affect our conclusions.

Experimental Section

A model free analysis on backbone NH rdcs has been performed on ubiquitin by Lakomek et al.[7]

The analysis of order parameters has been performed with Origin 6.1 and hydrogen bonds

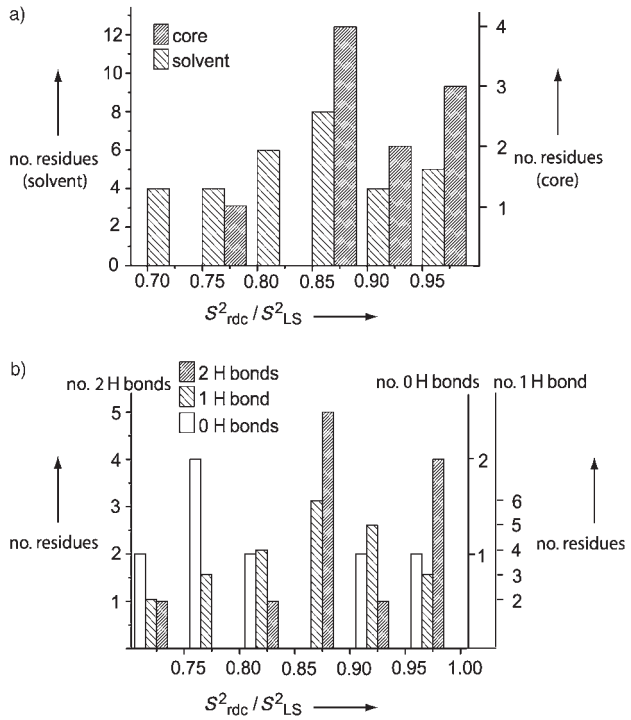

Figure 4. Histogram plot of the distribution of S^2_{rdc}/S^2_{LS} values. The residues are classified as core or solvent-exposed residues (a) or with respect to the number of backbone hydrogen bonds (b). The average S^2_{rdc}/S^2_{LS} order parameter is 0.91 ± 0.02 for core and 0.86 ± 0.02 for solvent-exposed residues. The values are 0.90 ± 0.02 for residues involved in two backbone hydrogen bonds, 0.86 ± 0.02 for one hydrogen bond, and 0.82 ± 0.05 for residues that are not involved in backbone hydrogen bonds. a) Core residues have a strong tendency for higher S^2_{rdc}/S^2_{LS} order parameters: 9 out of 10 core residues have a S^2_{rdc}/S^2_{LS} value greater than 0.85. b) Residues involved in two hydrogen bonds have higher S^2_{rdc}/S^2_{LS} values than those without any.

have been reported in the literature (see Supporting Information).[11,12] Figures 2 and 3 have been prepared with MOLMOL 2K.1 by using the ubiquitin structure (pdb 1d3z).

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Communications

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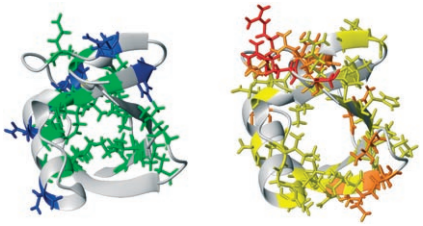

Order parameters derived from residual dipolar couplings between NH groups reveal motion of protein backbones on a time scale slower than the correlation time τ_C. Less-mobile amides (blue and green) in ubiquitin, for example, are H-bonded and belong to residues with side chains pointing towards the hydrophobic core while more mobile ones (yellow, orange, and red) have solvent-exposed side chains and fewer H bonds.