NMR Spectroscopic Investigation of ψ Torsion Angle Distribution in Unfolded Ubiquitin from Analysis of $^3J(C_\alpha,C_\alpha)$ Coupling Constants and Cross-Correlated $\Gamma^c_{H^NN,C_\alpha H_\alpha}$ Relaxation Rates

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The denatured state of a protein consists of an ensemble of conformers. Statistical models have been developed to describe the conformational averaging process for this dynamic state of peptides[1] and proteins.[2,3] Our model[2,3] assumes that all the interactions in a polypeptide chain studied at high concentrations of denaturant such as 8 M urea are local. Moreover, the distribution of conformers can be described by a statistical analysis of the distribution of torsion angles of residues in a database of native folded proteins not located in secondary structure elements. The Ramachandran diagram for all amino acids (Figure 1) shows that ϕ sampling is mainly restricted to values $-60° > \phi > -180°$, while ψ sampling covers $\psi \approx -30°$ and $100° < \psi < 180°$ of the diagram.

Heteronuclear NMR spectroscopy has been key to validate predictions based on our model. NMR studies revealed a remarkable correlation between predictions taken from the protein database and amino acid specific variations of the rotamer distribution around the angles ϕ[6] and χ_1[4] for the protein lysozyme denatured in 8 M urea at pH 2 as well as in studies of small unstructured peptides.[7]

For denatured ubiquitin a good correlation is observed between the predicted and the measured $^3J(H^N,H_\alpha)$ coupling constants averaged for a given residue type, for example, all alanines (Figure 2A and B). A set of 70 out of 73 possible $^3J(H^N,H_\alpha)$ coupling constants could be determined. This observation supports our model that predicts that conformational preferences in the denatured polypeptide chain are local and provides further evidence that the model does not depend on a specific peptide sequence.

However, since $^3J(H^N,H_\alpha)$ depend only on the angle ϕ, it is difficult to differentiate between two possible models of conformational averaging: a model in which ϕ,ψ sampling is restricted to positive ψ torsion angle space as opposed to a model in which the polypeptide chain is sampling both positive and negative ψ torsion angles. $^3J(H^N,H_\alpha)$ are indeed predicted to be similar if residues not located in secondary structure elements are taken into account that have positive ψ torsion angles only (Figure 2B) or that have both positive and negative ψ torsion angles (Figure 2A). The presence of experimental NOE contacts H^N_i, H^N_{i+1} and of $H_{\alpha i}$,H^N_{i+1} of similar intensity in denatured proteins indicate that averaging involves sampling of positive and negative ψ torsion angles, which is compatible with predictions of our model.[8]

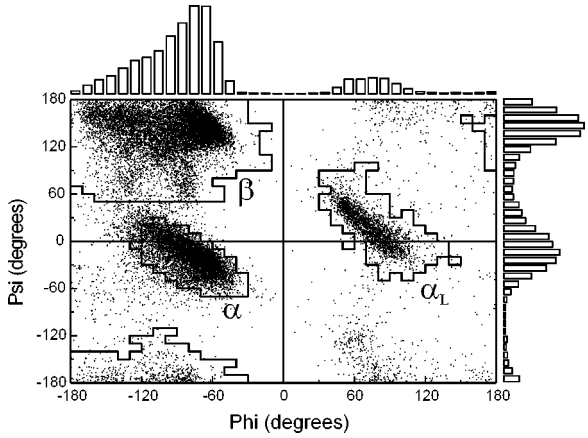

Figure 1. The distribution of ϕ,ψ torsion angles for all amino acids not located in secondary structure elements in a database of 402 high-resolution crystal structures of native folded proteins.[4] The α,β and α_L (combined α_{left} and γ_{left}) regions of ϕ,ψ space, as defined by Swindells et al. are labeled.[5]

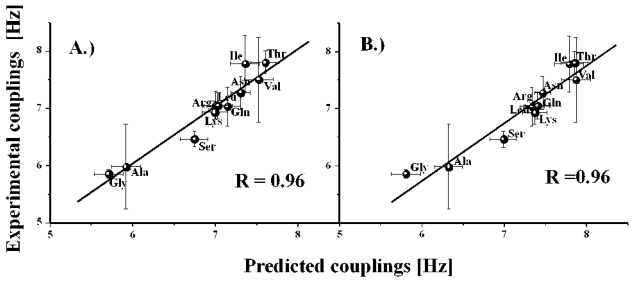

Figure 2. Correlation between experimental $^3J(H^N,H_\alpha)$ averaged for a given residue type, e.g., all alanines in ubiquitin denatured in 8 M urea, pH 2, and $^3J(H^N,H_\alpha)$ coupling constants predicted for amino acids excluding aromatic amino acids and Asp and Glu residues. Mean values for a given residue type are depicted as balls, error bars represent standard deviations. Three different Karplus parametrizations were used for the calculated couplings, error bars for predicted couplings represent standard deviations. (Complete information on experimental conditions and predictions is given in Table S3 in Supporting Information) (A) Comparison with predictions for residues that are not located in secondary structure elements in the database of native folded protein structures with ϕ,ψ torsion angles covering all regions of the Ramachandran diagram. (B) Comparison with predictions for residues that are not located in secondary structure elements and that have positive ψ torsion angles only.

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However, a quantitative analysis of populated rotamers from NOE intensities is difficult due to the r^{-6} averaging and complex dynamical properties that are potentially nonuniform along the denatured peptide chain.

In the present study, two NMR experiments were carried out to probe the conformational averaging around the protein backbone angle ψ. In particular, $^3J(C_\alpha,C_\alpha)$ coupling constants[9] and dipole, dipole cross-correlated relaxation rates[10−13] have been measured. The dependence of the cross-correlated relaxation rate $\Gamma^c_{H^NN,C_\alpha H_\alpha}$ between the N,H^N and $H_\alpha C_\alpha$ dipole tensors on their projection angle θ[10,11] is given in eq 1.1.

$$\Gamma^c_{H^NN,C_\alpha H_\alpha} = \frac{\gamma_H\gamma_N}{(r_{N,H^N})^3}\frac{\gamma_H\gamma_C}{(r_{C\alpha,H\alpha})^3}\left(\frac{\hbar\mu_0}{4\pi}\right)^2 \times \frac{1}{5}(3\cos^2\theta - 1)S^2\tau_c \quad (1.1)$$

For the native state of ubiquitin (10 mM phosphate, pH = 4.7, T = 303 K), in agreement with Pelupessy et al.,[13] large negative relaxation rates of −11.8 ± 1.8 Hz are observed for the β-sheet regions of the protein (Figure 3a, triangles). More positive relaxation rates are observed in α-helical and loop regions of the protein. For denatured ubiquitin, uniform cross-correlated relaxation rates with an average of −4.3 ± 1.8 Hz are observed (Figure 3a, balls). The structural interpretation of the cross-correlated relaxation rates for a dynamic system is difficult, since neither the overall correlation time τ_c nor the order parameter S^2 can be unambiguously determined for a denatured polypeptide chain. The ^{15}N T_1/T_2 ratios for residues 4−64 are 2.0 ± 0.3 indicating a relative uniform effective correlation time for the center part of denatured ubiquitin. However, the experimental cross-correlated relaxation rates in denatured ubiquitin are small and negative. The rates for residues 23−34 that are in the α-helix in the native state are more negative in the denatured state but do not reach the values found for the β-sheet region of the native state of ubiquitin. We interpret the more negative relaxation rates as a conformational averaging, in which residues sample sample both positive and negative ψ torsion angles in the denatured state.

To further gain insight into the conformational averaging in denatured ubiquitin, $^3J(C_\alpha,C_\alpha)$ coupling constants shown in Figure 3B were measured and compared with data for native ubiquitin. $^3J(C_\alpha,C_\alpha)$ depend[9] on the angle ψ. This unexpected relation, which is in contradiction to a Karplus-type dependence on the intervening torsion angle ω, was found for data obtained for native ubiquitin (Figure 3B, triangles). The mean experimental coupling constants $^3J(C_\alpha,C_\alpha)$ for residues in β-sheet regions of native ubiquitin are 1.69 ± 0.1 Hz, while coupling constants are too small to be observed in α-helical regions. In denatured ubiquitin, $^3J(C_\alpha,C_\alpha)$ are larger than 0.7 Hz throughout the sequence including residues 23−34 that are located in the α-helical region in the native state (Figure 3B, balls). The mean $^3J(C_\alpha,C_\alpha)$ coupling constant is 0.85 ± 0.2 Hz with a pair wise rmsd of 0.1 Hz (Table S2, Supporting Information) for the 41 resonances that are sufficiently resolved to allow coupling constants to be determined. As an exception, large $^3J(C_\alpha,C_\alpha)$ are observed for Pro19 and Pro38. This might reflect a strong preference of prolines for ϕ,ψ torsion angles in the polyprolyl region of ϕ,ψ space ($\phi \approx -60°, \psi \approx +150°$) and potentially restricted ψ sampling.

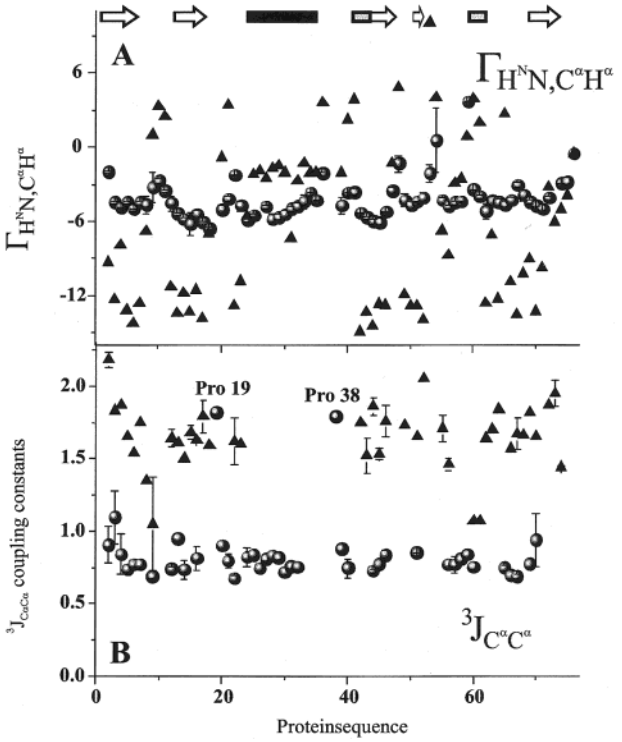

Figure 3. A. Cross-correlated relaxation rates $\Gamma^c_{H^N,C_\alpha H_\alpha}$ and B. coupling constants $^3J(C_\alpha,C_\alpha)$ in Hz measured for native (triangles) and for denatured (balls) ubiquitin. Pairwise root-mean-square differences of coupling constant values, which could be measured twice for successive residues, are given as error bars. Error bars for relaxation rates represent standard deviations from repeated experiments. Secondary structure elements in the native state of ubiquitin are indicated. All experiments were performed at 600 MHz. Experiments were carried out as described by Hennig et al.[9] and Pelupessy et al.,[13] respectively.

In conclusion, the two NMR parameters show that in a denatured polypeptide chain, individual amino acids sample both positive and negative ψ torsion angles. This observation has consequences for our understanding of the overall shape and tumbling of a polypeptide chain denatured in high concentrations of denaturant. The experiments reported here provide complimentary tools to study the nature of non-native states of proteins and deviations found in intermediate states by heteronuclear NMR spectroscopy.

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Supporting Information Available: A listing of measured coupling constants, cross-correlated relaxation rates of denatured ubiquitin, predictions of averaged coupling constants for different Karplus parametrizations and error analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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