Temperature Dependence of Fast Dynamics in Proteins

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ABSTRACT The temperature dependence of the internal dynamics of recombinant human ubiquitin has been measured using solution NMR relaxation techniques. Nitrogen-15 relaxation has been employed to obtain a measure of the amplitude of subnanosecond motion at amide N-H sites in the protein. Deuterium relaxation has been used to obtain a measure of the amplitude of motion of methyl-groups in amino-acid side chains. Data was obtained between 5 and 55°C. The majority of amide N-H and methyl groups show a roughly linear ($R^2 > 0.75$) temperature dependence of the associated Lipari-Szabo model-free squared generalized-order parameter (O^2) describing the amplitude of motion. Interestingly, for those sites showing a linear response, the temperature dependence of the backbone is distinct from that of the methyl-bearing side chains with the former being characterized by a significantly larger Λ-value, where Λ is defined as $d\ln(1 - O)/d\ln T$. These results are comparable to the sole previous such study of the temperature dependence of protein motion obtained for a calmodulin-peptide complex. This suggests that the distinction between the main chain and methyl-bearing side chains may be general. Insight into the temperature dependence is gathered from a simple two-state step potential model.

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The distribution of conformational states that a protein can occupy is potentially astronomical. Yet the precise nature of this ensemble of states can profoundly influence protein stability, dynamics, and ultimately function (1). Critical to this view is an understanding of the energy landscape between discrete conformational states. This is generally difficult to probe in comprehensive detail, i.e., in a site-resolved way throughout the protein of interest. Nuclear magnetic resonance (NMR) offers many avenues to the characterization of a variety of dynamic phenomena in proteins at atomic resolution. Particularly powerful are approaches based on solution NMR relaxation phenomena, which can allow the characterization of motion on the ps-ns (2,3) and μs-ms (4) timescales.

Previously we have examined the temperature dependence of subnanosecond dynamics of the main chain and methyl-bearing amino-acid side chains in calmodulin complexed with a peptide mimic of the calmodulin-binding domain of the smooth muscle myosin light chain kinase (5,6). The complex showed a wide range of temperature dependencies, with most sites showing a linear response consistent with a relatively simple effective potential of motion. Some sites showed a more complex temperature dependence indicative of a more complicated potential. In some of these latter cases, the perhaps counterintuitive nature of the temperature dependence strongly suggested the presence of conditional fluctuations arising from steric interactions (5).

The study of the calmodulin complex represents the only case where the temperature dependence of methyl-bearing side-chain motion has been studied in detail using deuterium relaxation methods. Thus, it is of interest to determine whether the dynamical features observed in that complex are present generally in proteins. Here we present a comparison of the temperature dependence of amide N-H and methyl-bearing, amino acid side-chain dynamics in the otherwise well-characterized protein ubiquitin.

Recombinant human ubiquitin was expressed during growth on appropriately isotopically enriched minimal media and purified as described previously (7). ^{15}N T_1, T_2, and the heteronuclear ^{1}H-^{15}N NOE relaxation parameters were measured at five temperatures (5°C, 15°C, 25°C, 45°C, and 55°C) at 11.7 T and 14.1 T, using the methods described elsewhere (8). ^{2}H relaxation rates of methyl CH_2D groups were measured from three multiple coherence relaxation experiments for I_zC_z, $I_zC_zD_z$ and $I_zC_zD_y$ (9) obtained at 11.7 T and 14.1 T and were acquired at six different temperatures (5°C, 15°C, 25°C, 35°C, 45°C, and 55°C). Relaxation data were analyzed in the context of the Lipari-Szabo model-free spectral density (10) with an in-house program based on an exhaustive grid-search algorithm (11). The fits employed an effective N-H bond length of 1.04 Å, an ^{15}N chemical shift tensor breadth of 170 ppm, and a deuterium quadrupolar coupling constant of 170 kHz. Reliability in obtained model-free generalized order parameters (O^2) and effective correlation times (τ_e) were estimated by Monte Carlo methods. See Marlow and Wand (12) for further details of typical sample preparation, data collection, and analysis.

The overall tumbling of the ubiquitin molecule was found to be effectively isotropic within the precision of the ^{15}N relaxation data used to characterize it. Correlation times of 8.84 ns (5°C), 6.36 ns (15°C), 4.71 ns (25°C), 3.58 ns

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(35°C), 2.77 ns (45°C), and 2.17 ns (55°C) were obtained. An excellent linear correlation between the obtained correlation time for macromolecular tumbling (τ_m) and η/T is seen across the full temperature range, consistent with the simple Stokes-Einstein relation for the reorientation of a sphere (using pure water viscosities; $r^2 = 0.999$; intercept, 0.33×10^{-9} s; slope, 1.66×10^{-9} K μPa^{-1}).

The squared generalized order parameters of 33 main-chain amide N-H bond vectors showed a linear ($R^2 > 0.7$) and >30% relative error in the slope in the correlation with temperature. For this group, the temperature coefficient ($\sigma_{NH} \equiv dO^2_{NH}/dT$) averaged $-(2.3 \pm 0.95) \times 10^{-3}$ K^{-1}. The squared generalized order parameters of the methyl group symmetry axis (O^2_{axis}) of 31 methyl groups showed a linear ($r^2 > 0.7$) dependence upon a variation in temperature (Fig. 1). This is consistent with a variety of simple potential energy functions governing the underlying motion (6). For this group, the temperature coefficient ($\sigma_{axis} \equiv dO^2_{axis}/dT$) averaged $-(2.6 \pm 1.1) \times 10^{-3}$ K^{-1}.

Recently, Palmer and co-workers have illuminated a fundamental prediction of the temperature dependence of the simple harmonic (quadratic) potential energy function to which an NMR spy might be attached (13). Vugmeyster et al. (13) demonstrated that the quantity Λ,

$$\Lambda = \frac{d\ln(1-O)}{d\ln T},$$

is ≤ 1 for a simple quadratic harmonic oscillator. It has been demonstrated in several cases that the values of Λ generally exceed this limit for amide N-H vectors. Here, we find the Λ-values for amide N-H sites in ubiquitin average 7.1 ± 2.6 (Fig. 2). The average Λ-value for methyl groups in ubiquitin is significantly lower (2.26 ± 1.0) but still exceeds the upper limit for the simple harmonic oscillator. The distribution is also somewhat narrower than that seen for the backbone amide N-H (Fig. 2). These values and observations are in accord with the temperature dependence of methyl dynamics in a calmodulin-peptide complex (5,6), the only other deuterium NMR relaxation study of the temperature dependence of methyl dynamics in proteins. The concordance between the two widely different protein systems begins to suggest that the distinction between the main chain and the methyl-bearing side chains may be a general property of proteins.

Simple models, such as motion within a harmonic or mildly anharmonic (e.g., quartic) potential, yield a smooth, almost linear temperature dependence of the order parameter but they cannot reproduce the large Λ-values seen experimentally (5). To gain insight into the energetics underlying the temperature dependence of the fast (subnanosecond) fluctuations, we introduce a simple azimuthally symmetric yet very anharmonic model in which the potential energy changes sharply for motion beyond a certain angular window (Fig. 3). The step potential is characterized by a potential of

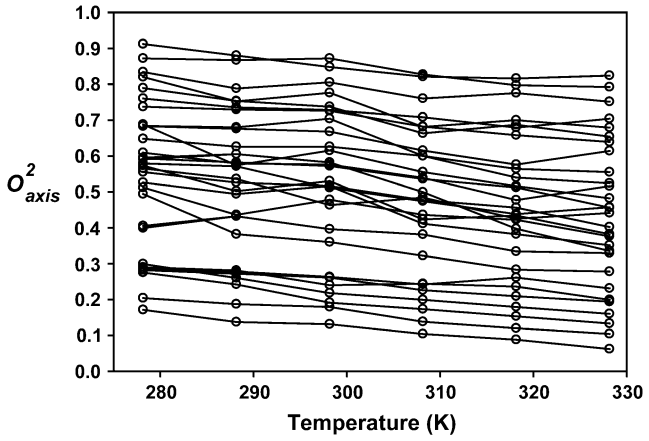

FIGURE 1 Temperature dependence of the amplitude of motion of methyl-bearing amino-acid side chains in ubiquitin. Shown are the model-free squared generalized order parameters obtained at 31 methyl sites using deuterium relaxation methods. Error bars have been omitted for clarity. On average, the precision of obtained generalized order parameters is estimated by Monte Carlo analysis to be ±0.03.

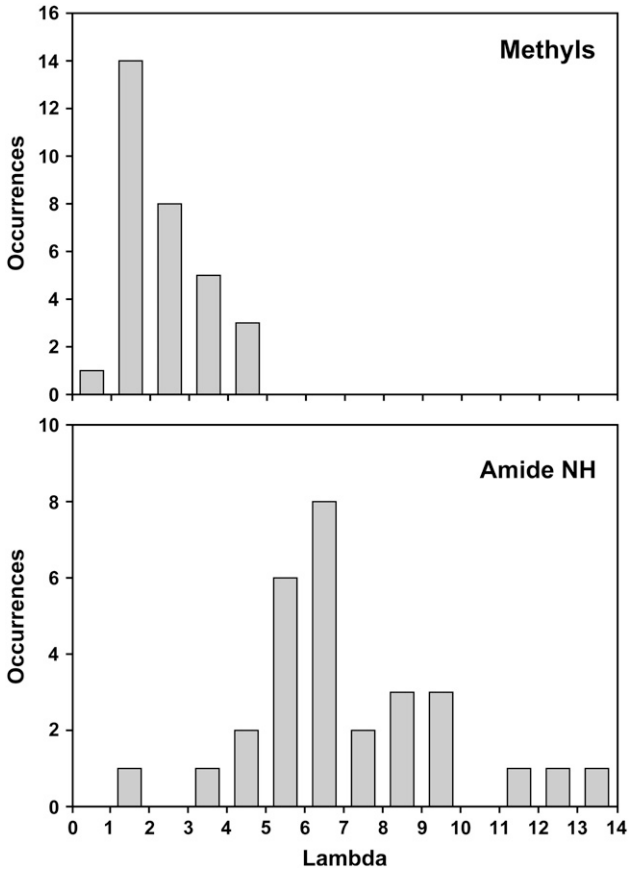

FIGURE 2 Distribution of λ-values for methyl symmetry axes (*top panel*) and amide N-H bond vectors (*bottom panel*) for sites having squared generalized order parameters showing a roughly linear temperature response.

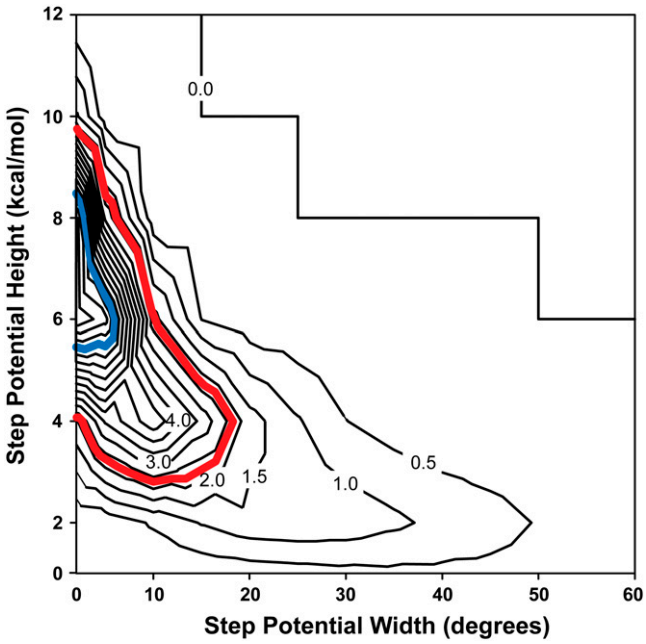

FIGURE 3 Predicted dependence the Λ-value for motion through an azimuthally symmetric step-well potential. Contours of the mean Λ-values for the backbone N-H and methyl symmetry axis observed in ubiquitin are colored blue and red, respectively.

U_0 for motion $|\theta|\langle\theta_0$, and U_1 for $|\theta|\rangle\theta_0$. Since only differences in potential matter, we can set $U_0 = 0$ to yield a two-parameter model, of width (θ_0) and step height (U_1).

The Λ-values corresponding to a range of step heights and angular widths are easily calculated for this model (Fig. 3). As might be expected, it is found that amide N-H and methyl symmetry axis Λ-values largely segregate.

The high Λ-values observed for the amide N-H in ubiquitin and in other systems (e.g., (5,13)) are most consistent with fluctuations across a step potential width of ~10° with a barrier height of 5–9 kcal/mol. It is difficult to ascribe this apparent barrier to a single factor such as hydrogen bonding or local steric interactions. However, such small fluctuations would not correspond to breakage of a hydrogen bond. It is also important to note that an intrinsic temperature dependence of the effective potential can also elevate Λ-values (14).

The methyl symmetry axis Λ-values circumscribe the step potential parameter space corresponding to the amide N-H Λ-values (Fig. 3). However, there is direct experimental evidence that low O^2_{axis} parameters reflect large angular excursions involving rotamer interconversion (2,5,15). The lower Λ-values observed for the methyl groups in ubiquitin and the calmodulin complex (5,6) are therefore most consistent with larger angular excursions and lower barrier heights (Fig. 3). Overall, these results are indicative of a view where the polypeptide chain acts as a relatively rigid and highly constrained scaffold while the attached (methyl-bearing) side chains are less restrained, more liquid-like, and moving across smaller barriers.

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