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Freezing of Dynamics of a Methyl Group in a Protein Hydrophobic Core at Cryogenic Temperatures by Deuteron NMR Spectroscopy

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Proteins undergo a number of significant changes when their temperature is dropped from the physiological range to much lower values. A so-called glass transition, occurring at ~200−230 K, is one of the most studied dynamical changes undergone by proteins in a solid state. This dynamic transition leads to a loss of biological activity[1,2] as a result of freezing of slow collective modes of motions, as evidenced from X-ray diffraction, neutron scattering studies, dielectric spectroscopy, and NMR relaxation measurements.[3−8] A number of works have presented arguments that connect the transition to solvent participation.[1,4,8−10] Solvent-related modes are frozen below the glass-transition temperature, and the dynamics at temperatures between 200 and 100 K is likely to be dominated by methyl group dynamics, which manifest themselves as anharmonic modes in neutron scattering measurements.[2,5,7] Several NMR studies have emphasized the importance of these modes in low temperature dynamics.[7,11,12] One of the latest works on the subject by Bajaj et al.[11] has reported a structural transition associated with dynamic processes in a solvent-free polypeptide. Thus, protein dynamics at low temperatures are complex, and more studies are required to discern their pattern.

For a precise quantitative description of changes experienced by proteins at low temperatures, it can be useful to look in detail at selected sites located at key positions of protein structures. While such an approach loses the breadth of the global study in which all methyl side chains are included, it allows for the level of quantification at a selected site which often cannot be achieved in multiple-labeled samples due to signal resolution and spin-diffusion issues in NMR studies.[12] The resulting data have a high potential to be useful for mechanistic explanations of observed phenomena.

We look at a hydrated powder sample of chicken villin headpiece subdomain(HP36),[13−17] labeled at a single methyl group of a key hydrophobic core residue L69 (Supporting Information S1). The details of sample preparation and sample conditions are given in S2. Our previous deuteron NMR studies on this system, conducted in the temperature range 298−140K, has suggested the presence of a crossover temperature of 170 K, at which the dynamics of the methyl group undergoes a dramatic change probably stemming from changes in the methyl environment.[18] This study reports the results of the temperature regime between 140 and 4 K in which we observe a direct indication of freezing of the methyl group dynamics at 95 K.

Deuteron solid-state NMR techniques, known to be very sensitive probes of molecular dynamics,[19−21] are used here to probe the dynamics of the methyl groups. In particular, we employ longitudinal relaxation time (T_{1Z}) measurements with a multiple-echo detection scheme (quadrupole Carr−Purcell−Meiboom−Gill pulse train, QCPMG) for signal-to-noise enhancement.[22,23] A static

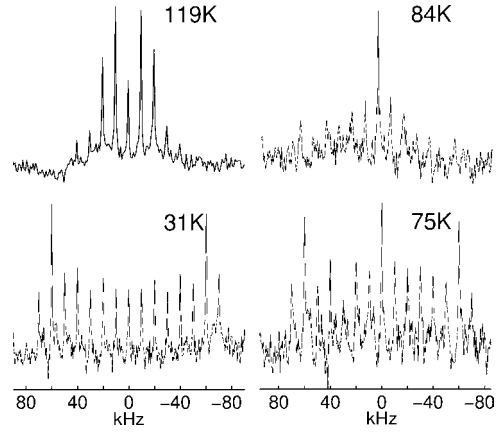

Figure 1. Deuteron QCPMG line shapes, taken from the most relaxed spectra of the T_{1z}-experiment, at three motional regimes. 119 K fast, 84 and 75 K intermediate, and 31 K slow motional regimes. All intensities are normalized. Number of scans was 1024, 3040, 512, and 128, respectively.

cryogenic helium-cooled probe[24] was indispensable for measurements down to 4 K. Details of the experimental procedure are available in S2.

Three-site hops of the methyl groups determine relaxation rates at the temperatures at which the system can be treated as classical. As evident both from the analysis of the T_{1Z} times and spectral line shapes (see Figure 1 and S3), the three-site hops undergo a switch between three motional regimes in the range of temperatures studied. In the fast regime, from 140 K down to ~100 K, the rate constant of the three-site hops k is much larger than the quadrupolar coupling constant C_q; in the intermediate regime, ~100−70 K, k is on the order of C_q; and in the slow regime, less than 70 K, k is much less than C_q. Relaxation times reported in Figure 2 were taken at the frequencies of maximum intensity. Relaxation anisotropy (i.e., the change of relaxation times as a function of frequency in the spectrum) is small, and the results are practically the same when the intensity of all QCPMG spikes is used in the calculation of the relaxation times. Three-site hop rate constants were extracted from T_{1Z}-QCPMG measurements using the EXPRESS[25] simulation program for the temperature range 140−61 K (S3). Line-shape simulations of the intermediate regime spectra suggest the presence of minor conformers in exchange with the major form. The fraction of the minor conformers can be fixed at 1.4% at 75 K. The details of the line-shape simulations are given in S4.

One of the striking features of the temperature dependence of the relaxation times is a sharp increase in the slope at ~95 K (Figure 2 insert), signaling a large rise in the apparent activation energy value. The activation energy changes from 4.7 ± 0.2 to 10 ± 2 kJ/mol. This indicates an abrupt freezing of methyl dynamics. As now commonly believed, methyl group dynamics is responsible

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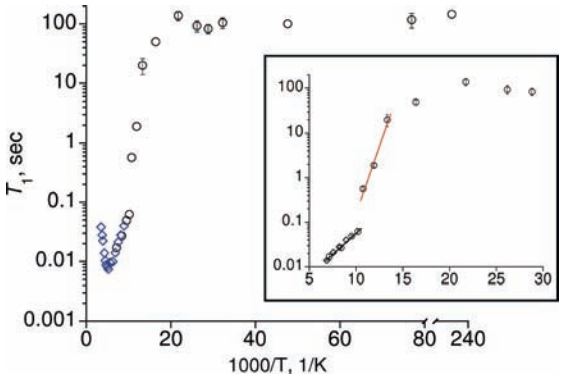

Figure 2. Deuteron T_{1Z} relaxation times at 11.7T versus 1000/T. The insert shows an expanded plot for the 140 K to 50 K range. Solid lines represent the best Arrhenius fit for either the 160−98 K temperature range (black) or 98−75 K temperature range (red). Diamonds represent the data collected via quadrupolar-echo detection scheme in a previous study. Circles represent the data collected via the multiple-echo detection scheme. Error bars which fall within the width of the symbols are omitted.

for the onset of anharmonicity observed in neutron scattering experiments.[2,7] However, neutron scattering is not a direct probe of site specific methyl group behavior, and usually its resolution cannot give information on their dynamics below 100 K due to a decrease in the rate constant k. Thus, NMR studies are unique in this aspect because they can give more information regarding the sources of the changes in the rate constants and provide a basis for detailed mechanistic interpretations. In an earlier work Andrew et al.[26] have looked at the dynamics of nonexchangeable protons in several polycrystalline proteins by solid-state NMR down to 10 K. They have not observed any changes in the activation energy values at ∼100 K. However, their measurements reflected an average behavior of all nonexchangeable protons in a protein molecule. Such a system has an inherent broad distribution of activation energies, and thus one cannot discern site-specific behavior from these measurements.

The biophysical nature of the observed change in the apparent activation energy of L69 in HP36 will be a subject of future investigations. As shown in the previous study,[18] at 170 K the apparent activation energy decreases 3-fold from its high temperature value. It remains to be seen whether the changes in the values of the activation energies at 170 and 95 K have a common source. Site-specific data for multiple locations in the hydrophobic core of native and mutant HP36 samples will be necessary to establish a mechanistic interpretation of these changes. It will thus provide information on whether the phenomena are typical for methyl groups buried in the core, depend on the type of the side chain, or require the presence of certain structural features in proximity to the methyl group.

Below 61 K the T_{1Z} temperature dependence curve deviates from the Arrhenius type behavior (shown by solid lines). At these temperatures, classical three-site hops become relatively ineffective contributors to relaxation and deuteron tunneling in a three-well potential gradually becomes a dominant mechanism.[27] The contribution of the tunneling to the relaxation rate manifests itself through phonon-induced transitions between torsional levels, mainly between ground and first excited states.[28,29] The energy differences between torsional states thus become a driving factor in the rise of the relaxation times at temperatures at which tunneling is the dominant mechanism. Figure 2 shows that for L69 in HP36 the relaxation times level out at low temperatures to ∼150 s. The largest possible slope that can match the data between 31 and 4 K sets the upper limit on the characteristic energy difference between the torsional levels at $\sim k_B \cdot 5$ K.

In conclusion, single site labeling in conjunction with deuteron T_{1Z}-QCPMG NMR measurements on a cryogenic probe have allowed for the observation of freezing of methyl group dynamics at ∼95 K for a key hydrophobic core methyl group in the HP36 protein, as evidenced by an abrupt increase in the apparent activation energy. Relaxation times at temperatures below 60 K are dominated by the deuteron tunneling mechanism.

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Supporting Information Available: S1. Ribbon diagram of HP36 with key hydrophobic core side chains displayed. S2. Details of experimental procedure and sample conditions. S3. Three-site hop rate constants obtained via EXPRESS simulations of T_{1Z}-QCPMG data for 140−61 K temperature range. S4. QCPMG line-shape simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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