Published in final edited form as:

J Phys Chem B. 2012 January 12; 116(1): 555–564. doi:10.1021/jp208966k.

Thermal hysteresis in the backbone and side chain dynamics of the elastin mimetic peptide [VPGVG]₃ revealed by ²H NMR

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Abstract

We report on experimental measurements of the backbone and side chain dynamics of the elastin mimetic peptide [VPGVG]₃ by ²H NMR echo spectroscopy and 2D T_1 - T_2 correlation relaxometry. The T_1 and T_2 relaxation times of the Gly α -deuterons and Val α -, β - and γ - deuterons of a hydrated sample reveal a thermal hysteresis when the temperature is raised from -10°C to 45°C and then subsequently cooled back to -10°C . In addition, near 30°C we observe a reduction in the slope of the $T_1(T)$ and $T_2(T)$ heating curves, indicating a structural change that appears to be correlated well to the known inverse temperature transition of this peptide. The thermal dependence of the correlation times of the Gly α -deuterons are well fit by an Arrhenius Law, from which we have measured $E_{act} = (20.0 \pm 3.1)$ kJ/mol when the sample is heated, and $E_{act} = (10.9 \pm 2.8)$ kJ/mol when cooled. Molecular dynamics simulations support the notion that the measured activation energy is determined largely by the extent of localized water, which is observed to decrease with increasing temperature from approximately 25°C to 42°C .

Introduction

Elastin is an intriguing protein that provides vertebrate tissues with their elastomeric properties.^{1–4} The elasticity of this remarkable protein is known to hinge on a complex interaction with the solvent; elastin is only elastic when hydrated.⁵ For example, experimental measurements of the Young's modulus demonstrated a dependence of the stress-strain relationship on solvent polarity.^{6–8} A well known characteristic of elastin is that it undergoes an inverse temperature transition at approximately 20°C to 40°C , a process that results in a reduction in the protein radius of gyration, expulsion of water and formation of a complex network of hydrogen bonds.^{1,9} It has been experimentally demonstrated that the repeating elastin motif [VPGVG]_{*n*} undergoes an inverse temperature transition, and lends itself as a simplified model of the more complex protein.^{9–12} Simulation studies have demonstrated that the physical signatures of the inverse temperature transition of [VPGVG]₁₈ include a decrease in the radius of gyration of the α -carbons, number of localized water molecules and number of peptide-water hydrogen bonds as the temperature is increased from approximately 20°C to 40°C .¹³ In addition, as the temperature is increased over this range, there is an observed increase in the number of peptide side-chain contacts and number of peptide-peptide hydrogen bonds. A variety of structural studies of elastin have been performed that have demonstrated the presence of twisted-rope like organization as well as the presence of α -helical and β -turn conformations.^{1,14–17} The type II β -turn was observed in simulations of a [VPGVG]₁₈ peptide¹³ and in experimental studies of [(VPGVG)₄(VPGKG)]₃₉¹⁸ and [VPGVG]₃¹⁹ using Nuclear Magnetic Resonance (NMR). Moreover, the dynamical properties of short elastin mimetic peptides appeared to hinge on a

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complex interaction with the solvent.²⁰ In our work the backbone and side chain dynamics of the elastin mimetic peptide [VPGVG]₃ are studied using ²H quadrupolar echo spectroscopy²¹ and 2D T_1 - T_2 correlation NMR.²² The experimental data reveal a thermal hysteresis of the T_1 and T_2 relaxation times as the sample is heated from -10°C to 45°C and then subsequently cooled. This observation appears to be correlated to that observed by other experimental schemes.²³ In addition, the data show a reduction in the slope of the $T_1(T)$ and $T_2(T)$ heating curves near 30°C, indicating a structural change that appears to be in good agreement with the notion of an inverse temperature transition.

Quadrupolar echo spectroscopy is a well known experimental technique that allows for studying the structure and dynamics of selectively deuterium labeled sites of a broad range of materials.²¹ The NMR line shape in this experimental scheme is determined largely by the quadrupolar Hamiltonian. The coupling between the nuclear quadrupolar moment and electric field gradient is affected by the orientation and motion of the labeled segment. A deuterium line shape can be analyzed in terms of restricted anisotropic motion by comparing the experimental data with a simulated spectrum.^{24,25} Quadrupolar echo experiments probe the large dynamic range of 10⁻⁷s to 10⁻³s. The molecular motion of side chains, for instance, have been extensively studied by deuterium NMR in a broad range of systems, providing detailed information relating to structure and dynamics (see Ref²⁶⁻³³ for example). The 2D T_1 - T_2 correlation NMR technique is a powerful tool to study the dynamics of multi-site systems.^{22,34,35} By implementing a 2D Inverse Laplace Transform (ILT), the T_1 and T_2 relaxation times of a site are correlated and manifested into a peak in a 2D map. The T_1/T_2 ratio obtained from the 2D T_1 - T_2 correlation map may be used to study the mechanism of relaxation in porous media.^{36,37} This method has recently been employed to study porous properties of water saturated sedimentary rocks,³⁸ in potato tissue,³⁹ in cement pastes⁴⁰ and in the study of exchange of water in hydrated elastin.⁴¹

Materials and Methods

Two deuterium labeled [VPGVG]₃ peptide samples were synthesized by California Peptide and New England Peptide using methods discussed in.¹ On one sample, denoted [VPG*VG*]₃, all Gly α -protons were substituted with ²H. In the second sample, denoted [VPGV*G]₃, the Val α -, β - and γ -protons on Val4, Val9 and Val14 were substituted with ²H. Both peptides were terminated by the acetyl and amine group at the C- and N-termini, respectively. The measured purity of the samples was >99.0% and the mass was within 0.1% of expected molecular weight. Deuterium NMR experiments were performed on lyophilized and H₂O hydrated peptides, using a 200MHz Tecmag Apollo NMR system and a home-built NMR probe. A two-pulse quadrupolar echo pulse sequence was employed to study the ²H NMR spectrum of the lyophilized peptides with a phase cycling scheme that suppresses artifacts due to short echo times.⁴² Quadrupolar echo spectra were simulated with WEBLAB.²⁴ The $\pi/2$ radio frequency pulse width in the quadrupolar echo experiment was 2.2 μ s, the inter-pulse spacing was set to 35 μ s and 26640 scans were accumulated with a recycle delay of 3s. For the water hydrated peptides, a ²H 2D T_1 - T_2 NMR experiment was implemented and the T_1 and T_2 NMR relaxation times were obtained by a 2D ILT of the data.³⁴ In these experiments, the $\pi/2$ pulse width was 15.9 μ s, the inter pulse echo time was 180 μ s, 15 points in the T_1 dimension were acquired with 624 scans and a recycle delay of 4s. All of our lyophilized samples were sealed in a 1.7 mm OD capillary tube with the sample size of approximately 5.0mm³. The samples were dissolved in water and placed in a capillary tube at a concentration of approximately 0.1g/ml. Molecular Dynamics (MD) simulations were performed on the [VPGVG]₃ peptide, using the OPLS-AA/L force field model⁴³ and a Berendsen thermostat⁴⁴ in GROMACS.⁴⁵ In the MD simulations, the peptides were terminated by the amine (-NH₂) and carboxyl (-COOH) groups at the N- and C-termini respectively and were placed in a cubic box solvated with water using the SPC216

water model.⁴⁶ At all temperatures the starting peptide structures were linear chains. We performed a short simulation restraining the peptide position and equilibrated the pressure for 10ps before executing the full simulations. Full simulations were performed for a duration of 4ns at temperatures ranging from 5°C to 50°C. The dynamical properties of the peptide at all temperatures were determined by averaging over the last 1ns of the simulation results (i.e. from 3ns to 4ns). Structural equilibrium was verified by observing that the root mean square displacement of the C $_{\alpha}$ reached an equilibrated value after 2ns of simulation.

Results and Discussion

MD simulations of [VPGVG]₃

The Ramachandran maps of the valine residues are shown in Fig. 1, and various MD derived thermal characteristics of the peptide are tabulated in Table 1. It is clear from Fig. 1 that the α -helical structure becomes more prominent above 25°C than that at lower temperatures. The α -helical structure has also been observed in MD simulations of [VPGVG]₁₈¹³ and in the oligopeptide GVG(VPGVG).⁴⁷ Referring to Table 1, the simulations reveal a decrease in the radius of gyration, as well as an increase in the number of side-chain contacts and number of peptide-peptide hydrogen bonds between 25°C to 35°C. In addition, the simulations reveal a decrease in number of water molecules within 0.35nm of the peptide and number of peptide-water hydrogen bonds between 25°C to 35°C indicating that water molecules are expelled as the temperature is raised. Our 4ns simulation findings of the [VPGVG]₃ peptide, tabulated in Table 1, appear to be similar to that observed in short 9ns simulations of [VPGVG]₁₈.¹³ Fig. 1 also highlights the presence of a type II β -turn at all temperatures studied. The observation of a type II β -turn has also been made in simulation studies of [VPGVG]₁₈ at 10°C and 42°C¹³ and has been experimentally observed in a [(VPGVG)₄(VPGKG)]₃₉ peptide by NMR chemical shift analysis¹⁸

NMR studies of lyophilized [VPG*VG*]₃ and [VPGV*G]₃

Using the quadrupolar echo pulse sequence, ²H NMR spectra of lyophilized [VPG*VG*]₃ were obtained at various temperatures and are shown in Figs. 2A through 2E. In addition, a similar experiment was also performed on the water hydrated [VPG*VG*]₃ peptide at -30°C, and the result of this experiment is shown in Fig. 2F. In the experiment on the water hydrated peptide, no pake pattern was observed above -30°C. To be clear, above -30°C the broad pake pattern shown in Fig. 2E on the water hydrated sample was not observed, but a narrow Lorentzian was measured (with a line width of $1/\pi T_2$) as discussed in the next section. This finding may suggest that the motion of the Gly α -deuterons at -30°C is quenched due to freezing of localized water.

The experimental line shapes shown in Fig. 2 were simulated in WEPLAB with a model involving two-site jumps of the Gly α -deuterons about the axis indicated by an arrow in Fig. 3 with $\theta=55.7^\circ$ and $\phi=180^\circ$. In the model, θ was measured from the axis labeled in Fig 3, and ϕ was the relative phase between the two deuterons. All other motions were assumed very slow and therefore ignored. The simulated spectra are also shown in Fig. 2 and the best fit parameters for the jump rate, ω_{rot} , are provided in Table 2. At all temperatures, the quadrupolar asymmetry parameter $\eta=0$ was assumed in the simulations. The temperature dependence of the quadrupolar frequency, $\omega_q/2\pi$, determined by the simulation are also tabulated in Table 2 and appears to be invariant with temperature. Together, the two observations on the lyophilized and water hydrated sample at -30°C reveal the dynamics of the Gly α -deuterons depend heavily on the extent of hydration as well as the mobility of the solvent. An analogous observation was made in the ¹³C NMR spectra of bovine nuchal ligament elastin.⁴⁸ Specifically, the study showed that the mobility of the aliphatic carbons depends dramatically on the extent of hydration and when the sample was frozen at -20°C

no changes in the spectra were observed over a broad range of hydration levels. Evidence of hydration dependent dynamics of an elastin mimetic peptide Poly(Lys-25), [VPGVG₄VPGKG]₃₉ has also been revealed by ¹³C NMR.²⁰ Below 20 percent hydration the the backbone motion of this mimetic peptide was observed to increase slightly, whereas when the peptide was hydrated above 30 percent both the side chains and backbone were observed to undergo large amplitude fluctuations.

²H NMR quadrupolar echo spectroscopy was also performed on the lyophilized [VPGV*G]₃ peptide and the spectra are shown in Figs. 4A to 4F. Fig. 4G highlights results from the water hydrated [VPGV*G]₃ peptide at -20°C. In the experiment on the water hydrated peptide, no pake pattern was observed above -20°C, and we believe that this observation may be correlated to the freezing of localized water near the Val deuterons, as in the case of the Gly deuterons. One possible reason that the motion of the Val deuterons in the hydrated sample appears to be hindered at -20°C rather than -30°C (which was observed in the case of the Gly α deuterons) may be due to steric hindrance of the Val side chain. In simulating the line shapes, a superposition of two spectra involving “fast” and “slow” motion about the C $_{\alpha}$ -C $_{\beta}$ axis (labeled χ_2 in Fig. 5) was assumed undergoing three-site jumps with $\theta=70.6^\circ$ and $\varphi=(0^\circ, 120^\circ, 240^\circ)$. The angle θ was measured from the axis labeled χ_2 in Fig 5, and φ was the relative phase of the Val C $_{\gamma}$ and β -deuterons sites. The decomposition of the line shape simulated at 45°C is shown in Fig. 6 as an example. At all temperatures, the Val α -deuterons were assumed static, and the quadrupolar asymmetry parameter $\eta=0$ was assumed for all the deuterons. The methyl groups were assumed to undergo fast motion with a rotation frequency >1MHz about the respective C $_{\beta}$ -C $_{\gamma}$ axis (labeled χ_1 in Fig. 5). The best fit parameters for the jump rate of the motions about the C $_{\alpha}$ -C $_{\beta}$ axis, ω_{rot} , are listed in Table 3, and the simulated spectra are shown in Fig. 4. In simulating the powder line shapes, we found a reasonable fit was achieved by assuming a quadrupolar frequency of 117kHz for the Val α - and β -deuterons, and an effective quadrupolar frequency of 38kHz for the Val γ -deuterons (the effective quadrupolar frequency results from the fast motion of the methyl group about the C $_{\beta}$ -C $_{\gamma}$ axis). Referring to Table 3, the “slow” motion of the Val γ - and β -deuterons about the C $_{\alpha}$ -C $_{\beta}$ axis is observed to be independent of the temperature studied, and is similar to the thermal behavior observed in the Gly α -deuterons of the lyophilized [VPG*VG*]₃ peptide. However the Val γ - and β -deuterons undergoing “fast” motion about the C $_{\alpha}$ -C $_{\beta}$ axis exhibit a temperature dependence, specifically, a 3-fold increase in mobility is observed above 25°C. In addition, Table 3 highlights the ratio of the contribution of the “fast” to “slow” motion to the total signal. It is observed that this ratio increases with increasing temperature, which may be attributed to side chain mobility. The requirement of two motions in the line shape fitting may be due to nonuniform packing, or due to dynamical heterogeneity of the three valine residues (i.e. Val4, Val9 and Val14) in the 15-residue peptide. The structural heterogeneity of the valine residues in hydrated [VPGVG]₃ is observed in our MD results, highlighted in Fig. 1, as well as in simulations of hydrated [VPGVG]₁₈.¹³ Specifically, one observes the presence of both a α -helix and type II β -turns. In addition, a structural heterogeneity of the valine residues was experimentally observed via solid state NMR in hydrated [LGGVG]₆, another repeating motif of elastin.⁴⁹

NMR studies of hydrated [VPG*VG*]₃ and [VPGV*G]₃

The T_1 and T_2 NMR relaxation times of the Gly α -deuterons in the water hydrated [VPG*VG*]₃ peptide were measured via the ²H 2D T_1 - T_2 correlation NMR technique. The resulting 2D map at 30°C is shown in Fig. 7A as an example. One peak that correlates the T_1 and T_2 relaxation times is observed in Fig. 7A, and similar features were revealed for all the temperatures studied. The observation of a single peak indicates that all the Gly α -deuterons of the hydrated sample have similar T_1 and T_2 values and therefore have indistinguishable dynamics. Fig. 8 highlights the Gly α -deuteron T_1 and T_2 relaxation times as a function of

temperature. The error bars shown in the Fig. 8 were estimated by considering the standard deviation of all measured T_1 and T_2 times in the 2D experiment. To be clear, we performed an exponential fits to the experimental data and used the derived standard deviation of the values determined from the exponential fits as estimates of the errors in the relaxation rates. We found that this method was similar to considering the width of the distributions in T_1 or T_2 dimensions in the 2D T_1 - T_2 correlation maps.

In this temperature dependent study, the sample temperature was first increased from -10°C to 45°C and then decreased back to -10°C ; the experiment at each temperature was performed for approximately 10 hours with an average of 5 hours of temperature equilibration between experiments; approximately 2 weeks time elapsed between the first and last experiment. A difference in T_1 and T_2 relaxation times between the heating and cooling cycles is revealed from Fig. 8, indicating a thermal hysteresis of the Gly α -deuteron dynamics. In addition, a decrease in the slope of $T_1(T)$ and $T_2(T)$ heating curves is observed near 30°C in Fig. 8. To be clear, Fig 8A shows a plot of the T_1 and Fig. 8B the T_2 versus temperature. In the heating curves (shown in blue circles) we observe a change in the slope of the relaxation times versus temperature near 30°C ; the slope appears to reduce. To give insight into the possible physical properties that may cause this reduction, we determined the number of water molecules within 0.35nm of the Gly α -protons by MD simulation. Fig. 9A highlights results from this computation—a reduction in the number of water molecules is observed near 30°C . The reduction of localized water near the Gly α -protons in simulation appears to be correlated to the observation of a change in the slope of the $T_1(T)$ and $T_2(T)$ heating curves revealed in the experimental data. The observed thermal hysteresis in the Gly α -deuteron dynamics may be due to structural changes that ensue from the inverse temperature transition even after the temperature is reduced.

According to the Bloembergen-Purcell-Pound (BPP) theory, the autocorrelation function of motion with a single correlation time is given by⁵⁰

$$K(\tau) = \langle F(t)F^*(t) \rangle e^{-|\tau|/\tau_c} \quad (1)$$

where τ_c is the correlation time, $F(t)$ is a function of position of spatial coordinates, and the $\langle \rangle$ sign represents an average over all possible coordinates. The spectral density is the Fourier transform of Eq. 1, and is given by

$$J(\omega) = \langle F(t)F^*(t) \rangle \frac{2\tau_c}{1 + (\omega\tau_c)^2} \quad (2)$$

where ω is the Larmor frequency. For ^2H nuclei, the NMR relaxation is governed by the quadrupolar interaction. The function $F(t)$ for the quadrupolar interaction is angular dependent and the averaging process depends on the anisotropy of the motion of the nucleus. An important point that should be made clear is that the average $\langle F(t)F^*(t) \rangle$ is a constant that multiplies the spectral density. The spectral density functions for the T_1 and T_2 relaxation times are given by⁵¹

$$\frac{1}{T_1} = \frac{3}{40} C_q \left\{ \frac{\tau_c}{1 + (\omega_D \tau_c)^2} + \frac{4\tau_c}{1 + 4(\omega_D \tau_c)^2} \right\} \quad (3)$$

$$\frac{1}{T_2} = \frac{1}{20} C_q \left\{ 9\tau_c + \frac{15\tau_c}{1 + (\omega_D \tau_c)^2} + \frac{6\tau_c}{1 + 4(\omega_D \tau_c)^2} \right\} \quad (4)$$

In Eq. 3 and 4, C_q is the quadrupolar interaction strength and ω_D is the Larmor frequency for ^2H . For isotropic motion C_q is given by

$$C_q = 4\pi^2 \left(1 + \frac{\eta^2}{3} \right) \left(\frac{e^2 q Q}{h} \right)^2 \quad (5)$$

where η is the asymmetry parameter of the ^2H electric field gradient and $\frac{e^2 q Q}{h}$ is the quadrupolar coupling constant. However, for anisotropic motion, C_q will be scaled by a constant. The motion of the nuclear spin in question at a particular site may be complex. In our analysis of the data, we have assumed a single correlation time to model the complex motion. Using Eq. 3 and 4, the τ_c of the Gly α -deuterons were determined from the measured T_1 and T_2 values for all the temperatures studied and are plotted in Fig. 10. To be clear, Eq. 3 and 4 are a system of two equations with two unknowns (τ_c and C_q) and determination of either parameter requires a measurement of both T_1 and T_2 relaxation times. With the sample temperature raised to 45°C or cooled from 45°C back to -10°C, the correlation time appears to fit well within the experimental uncertainties to an Arrhenius Law, from which we have determined $E_{act} = (20.0 \pm 3.1)$ kJ/mol when heated and $E_{act} = (10.9 \pm 2.8)$ kJ/mol when cooled for the Gly α -deuterons. The reduction in the activation energy of the Gly α -deuterons also appears to be correlated to the extent of exposure to water resulting from the structural changes that ensue from the inverse temperature transition.

We also performed the 2D T_1 - T_2 correlation experiments on the hydrated $[\text{VPGV*G}]_3$ peptide; an example of the 2D T_1 - T_2 map at 30°C is shown in Fig. 7B. Different from the 2D map of $[\text{VPG*VG*}]_3$ shown in Fig. 7A, two distinguishable peaks are revealed in Fig. 7B, indicating that there are two different relaxation times amongst all valine deuterons. The ratio of the signal intensities of the two peaks is measured to be approximately 6:2, and the larger intensity peak has larger T_1 and T_2 relaxation times corresponding to faster motion. The larger intensity peak is therefore attributed to the Val γ -deuterons (labeled Val $_\gamma$ in Fig. 7B) and the smaller intensity peak to the Val α - and β -deuterons (labeled Val $_{\alpha,\beta}$ in Fig. 7B). Similar features shown in Fig. 7B are observed for all the temperatures studied. In addition to the expected difference in mobility of the Val α - and β -deuterons and Val γ -deuterons, the assignment of the two signals in the 2D T_1 and T_2 map was further confirmed by investigating the line widths, relative intensities and chemical shifts of the ^2H spectra of the hydrated $[\text{VPGV*G}]_3$ peptide. The measured T_1 and T_2 relaxation times associated with different deuterons are plotted in Fig. 11 as a function of temperature. The error bars shown in the figure were estimate using a similar technique described previously for the Gly α -deuterons.

Referring to Fig. 11, the thermal hysteresis in relaxation times is also observed for all Val deuterons of the hydrated $[\text{VPGV*G}]_3$ peptide, but the effect appears to be smaller than that observed in the Gly α -deuterons shown in Fig. 8. Referring to Fig. 11, a change in the slopes of the $T_1(T)$ and $T_2(T)$ heating curves at approximately 30°C is observed for all the Val deuterons. To be clear, Fig 11 shows the T_1 and T_2 versus temperature for all Val deuterons. The heating curves (shown as blue circles), in all cases, show a reduction in slope near 30°C. Fig. 9B and 9C highlight MD simulation results of the number of localized water molecules

at the Val α - and β -protons and the Val γ -protons. The figures show a reduction in localized water near 30°C to 35°C at all sites, and the findings appear to be correlated to the experimental observation of a reduction in the slope of the $T_1(T)$ and $T_2(T)$ heating curves at this temperature.

The correlation times of Val γ -deuterons were determined by using Eq. 3 and 4 from the measured T_1 and T_2 values, and are shown in Fig. 12. Different from the correlation times of the Gly α -deuterons shown in Fig. 10, there is no measurable difference in correlation times of Val γ -deuterons, within our experimental uncertainty, as the sample temperature is varied. For lyophilized peptides one might expect that methyl correlation times would decrease with increasing temperature. For example, the jump rate of methyl groups in polycrystalline L-alanine were observed to approximately double with increasing temperature from -120°C to -70°C.⁵² In the hydrated [VPGVG]₃ peptide, the inverse temperature transition drives an increase in side-chain contacts and an expulsion of localized water. While the increase in temperature may normally increase side chain mobility, steric hindrance arising from the increase in side chain contacts, the reduction in radius of gyration and the reduction in localized water would reduce side chain motion. The competition of these mechanisms may be manifested in the temperature dependence of τ_c for the Val γ -deuterons shown in Fig. 12. Lastly, it is worth noting that the correlation time determined from the T_1 and T_2 values using Eq. 3 and 4 has a large uncertainty when T_1 and T_2 are approximately equal. The method used to determine τ_c therefore becomes challenging to implement for the Val α - and β -deuterons shown in Fig. 11C and 11D.

In addition to the observation of a thermal hysteresis reported here, the thermal hysteresis of the elastin like peptides poly(VPGVG) and poly(VPAVG) has been experimentally studied by differential scanning calorimetry and turbidity (by monitoring the change in absorbance at 300nm).²³ In their study, a clear hysteresis is evident in the calorimetry data over a time scale of approximately 10 minutes in the range of -5°C to 60°C as well as the turbidity over a range of approximately 7°C to 40°C. While these calorimetry and turbidity studies probe macroscopic behavior of the polypeptides in solution, the methods implemented herein have provided for site-specific, microscopic information relating to the hysteresis that may be relevant in light of continued efforts to design artificial, biologically inspired elastomers.^{53,54}

Conclusion

In this work we have investigated the valine and glycine backbone and side chain dynamics of the elastin mimetic peptide [VPGVG]₃ by ²H quadrupolar echo spectroscopy and 2D T_1 - T_2 correlation NMR. A key feature of the experimental data is the observation of a thermal hysteresis when the sample temperature is increased from -10°C to 45°C and subsequently cooled back to -10°C. Using an Arrhenius model, the measured activation energies of the Gly α -deuterons are (20.0 ± 3.1) kJ/mol when the sample is heated and (10.9 ± 2.8) kJ/mol when cooled. The observed reduction in the activation energy of the Gly α -deuterons appears to be correlated to a well known inverse temperature transition of the peptide; a process by which the peptide radius of gyration, number of localized water molecules and peptide-water hydrogen bonds reduce with increasing temperature from approximately 20°C to 42°C. In addition, the experimental data indicate that the measured correlation times of the Val γ -deuterons appear to be invariant with temperature. It is argued that the increase in number of side chain contacts and reduction of localized water counteract the thermally driven process that would otherwise increase the mobility of the Val γ -deuterons. Lastly, the experimentally measured $T_1(T)$ and $T_2(T)$ curves of all Val and Gly deuterons show a reduction in slope upon heating at approximately 30°C that appears to be correlated to the extent of localized water as demonstrated by MD simulation.

Acknowledgments

The authors thank Yi-Qiao Song of Schlumberger-Doll Research for allowing us to implement the ILT algorithm and Laura J. Juszcak, Lesley Davenport and Richard Magliozzo for useful discussions. G. S. Boutis acknowledges support from the Professional Staff Congress of the City University of New York and NIH grant number 7SC1GM086268-03. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health.

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Figure 1. Valine Ramachandran maps of water solvated [VPGVG]₃ peptide derived from MD simulation at (A) 10°C, (B) 20°C, (C) 25°C, (D) 30°C, (E) 35°C and (F) 42°C.

Figure 2. ^2H quadrupolar echo NMR spectra of the (A–E) lyophilized $[\text{VPG}^*\text{VG}^*]_3$ peptide and (F) H_2O hydrated $[\text{VPG}^*\text{VG}^*]_3$ peptide. (A) 37°C , (B) 25°C , (C) 10°C , (D) -5°C and (E) -20°C . The spectra shown in (F) was acquired at -30°C where it is believed that localized water near the Gly α -deuterons is frozen, as described in the text. A Gaussian broadening of 3kHz was applied to both simulation and experimental data. Experimental data are indicated in black lines and simulation results are shown by blue lines.

Figure 3. Sketch of the molecular structure of glycine. In the figure, the arrow defines the axis of rotation assumed in the model described in the text.

Figure 4. ^2H quadrupolar echo NMR spectra of the (A–F) lyophilized $[\text{VPGV}^*\text{G}]_3$ peptide and (G) H_2O hydrated $[\text{VPGV}^*\text{G}]_3$ peptide. (A) 45°C , (B) 35°C , (C) 25°C , (D) 10°C , (E) -5°C and (F) -20°C . The experimental data shown in (G) was acquired at -20°C where it is believed that the localized water near all the valine deuterons is frozen. A Gaussian broadening of 3kHz was applied to both simulation and experimental data. Experimental data are indicated in black lines and simulation results are shown by blue lines.

Figure 5. Sketch of the molecular structure of valine. The motion about χ_1 , is assumed to be fast (>1 MHz). As described in the text, a superposition of two motions for the Val γ - and β -deuterons is assumed to take place about the C $_{\alpha}$ -C $_{\beta}$ axis.

Figure 6.

Decomposition of the simulated ^2H NMR spectrum of the lyophilized [VPGV*G]₃ peptide at 45°C. (A) Spectra of the “fast” motion about the C_α - C_β axis of valine, (B) “slow” motion about the C_α - C_β axis of valine and (C) overall spectrum. In (A) and (B) dot-dashed lines are Val γ -deuterons, solid lines are Val α -deuterons and dashed lines are Val β -deuterons. Dashed lines are offset by -0.1 for clarity.

Figure 7.

2D ILT maps of the T_1 - T_2 correlation NMR experiments on (A) H₂O hydrated [VPG*VG*]₃ peptide at 30°C and (B) H₂O hydrated [VPGV*G]₃ peptide at 30°C. One peak (labeled Gly_α) is observed in (A) and two peaks (labeled Val_γ and Val_{α,β}) are observed in (B). The ratio of the two peaks shown in (B) is approximately 3.2.

Figure 8.

Experimental results of the ^2H (A) T_1 and (B) T_2 NMR relaxation times of the H_2O hydrated $[\text{VPG}^*\text{VG}^*]_3$ peptide as a function of temperature. In the experiments, the temperature of the sample was first increased from -10°C to 45°C (blue, open circles) and then decreased back to -10°C (red, filled squares). Each experimental data point took approximately 10 hours to acquire. The time between the first and last experiment was approximately 2 weeks. The estimation of the error bars is described in the text of the manuscript.

Figure 9.

MD derived simulation results highlighting the number of localized water molecules within 0.35nm of (A) Gly α -protons, (B) Val α - and β -protons and (C) Val γ -protons of the [VPGVG]₃ peptide. The error bars shown in the figure represent the statistical standard deviation over 1ns of simulation time.

Figure 10.

Measured correlation times (τ_c) of the Gly α -deuterons of the H₂O hydrated [VPG*VG*]₃ peptide, as a function of inverse temperature, T^{-1} . Blue, open circles represent the experimental data when the sample temperature was increased; red, filled squares indicate the data when the temperature was subsequently decreased. The solid lines are the best fits to $\tau_c = \tau_c^0 \exp(E_{act}/k_B T)$ where τ_c^0 is a constant, and the best fit parameters for the activation energy are $E_{act} = (20.0 \pm 3.1) \text{ kJ/mol}$ when heated and $E_{act} = (10.9 \pm 2.8) \text{ kJ/mol}$ when cooled. For the blue, open circles the $\chi^2/\nu = 0.88$ and for the red, filled squares $\chi^2/\nu = 0.23$, where χ^2/ν is the reduced chi-square of the fit. The error bars shown were propagated from the error bars in the measured T_1 and T_2 relaxation times.

Figure 11.

Experimental results of the ²H (A) Val γ -T₁, (B) Val γ -T₂, (C) Val α - and β -T₁ and (D) Val α - and β -T₂ NMR relaxation times of the H₂O hydrated [VPGV*G]₃ peptide as a function of temperature. In the experiments, the temperature of the sample was first increased from -10°C to 45°C (blue, open circles) and then decreased back to -10°C (red, filled squares). Each experimental data point took approximately 10 hours to acquire, and the time between the first and last experiment was approximately 2 weeks. The estimation of the error bars is described in the text of the manuscript.

Figure 12.

Measured correlation times, (τ_c), of the Val γ -deuterons of the H_2O hydrated $[\text{VPGV}^*\text{G}]_3$ peptide as a function of temperature (T). Blue, open circles represent the experimental data when the sample temperature was increased; red, filled squares indicate the data when the temperature was subsequently decreased. The error bars shown were propagated from the error bars in the measured T_1 and T_2 relaxation times.

Table 1

MD derived simulation results for [VPGVG]₃.

Temp (°C)	R _g ^a (nm)	Side-Chain Contacts ^b	Number of Water Molecules ^c	Peptide-Peptide Hydrogen Bonds ^d	Peptide-Water Hydrogen Bonds ^d
5	1.26 ± 0.06	143 ± 3	369 ± 26	0.03 ± 0.16	31.6 ± 2.7
10	1.98 ± 0.08	153 ± 5	365 ± 27	0.10 ± 0.31	30.2 ± 2.7
20	1.06 ± 0.05	143 ± 3	367 ± 28	0.02 ± 0.14	31.0 ± 2.9
25	1.01 ± 0.07	152 ± 7	342 ± 26	0.10 ± 0.31	29.7 ± 2.8
30	1.13 ± 0.08	142 ± 3	355 ± 26	0.14 ± 0.40	30.0 ± 3.0
35	0.79 ± 0.04	156 ± 6	324 ± 26	0.61 ± 0.65	27.5 ± 2.9
42	0.83 ± 0.07	158 ± 8	316 ± 28	0.35 ± 0.60	28.1 ± 2.9
50	0.63 ± 0.02	180 ± 11	289 ± 31	1.47 ± 1.01	24.0 ± 3.0

The values shown in the table were determined by performing computations over all residues of the peptide.

All values shown in the table were obtained by performing computations over the last 1ns of the MD simulation.

^aThe radius of gyration (R_g) was determined by computing the position of the C α .

^bSide-chain contacts were counted when two neighboring aliphatic carbon atoms from neighboring residues were within 0.54 nm of each other.

^cNumber of water molecules were counted when a water molecule was within 0.3nm of any peptide atoms.

^dHydrogen bonds were counted when the donor-acceptor cutoff distance of 0.35nm and the hydrogen-donor-acceptor angle of 30° was satisfied.

Table 2

Parameters used for simulating the ^2H NMR quadrupolar echo line shape of the lyophilized $[\text{VPG}^*\text{VG}^*]_3$ spectra shown in Fig 2. A two-site jump model was assumed about the axis shown in Fig. 3 of the Gly α -deuterons with $\theta = 55.7^\circ$ and $\varphi = 180^\circ$, as described in the text.

Temp ($^\circ\text{C}$)	$\omega_{rot}/2\pi$ (kHz)	$\omega_q/2\pi$ (kHz)
37	3	122
25	3	122
10	3	122
-5	3	122
-20	3	122
-30 (Hydrated)	3	120

Table 3

Parameters used for simulating the ^2H NMR quadrupolar echo line shape of the lyophilized $[\text{VPGV}^*\text{G}]_3$ spectra shown in Fig 4. A three-site jump model was assumed about the Val C_α - C_β axis shown in Fig 5 with $\theta = 70.6^\circ$ and $\varphi = (0, 120^\circ, 240^\circ)$. In the model, methyl rotors were assumed to undergo fast motion (with a rotational frequency > 1 MHz) about the respective C_β - C_γ axis labeled χ_1 in Fig. 5. A superposition of “fast” and “slow” motion of the Val γ - and β -deuterons was assumed with rotational frequencies indicated by the second and third columns, respectively. The contribution of the fast and slow motion to the total signal is indicated by the last column. As described in the text, the Val α -deuterons were assumed static at all temperatures.

Temp ($^\circ\text{C}$)	Slow $\omega_{\text{rot}}/2\pi$ (kHz)	Fast $\omega_{\text{rot}}/2\pi$ (kHz)	Val γ -deuterons $\omega_q/2\pi$ (kHz)	Val α - and β -deuterons $\omega_q/2\pi$ (kHz)	Ratio of “fast” to “slow” motion
45	3 to 4	100	38	117	1.8
35	3 to 4	100	38	117	1.4
25	3 to 4	30	38	117	0.8
10	3 to 4	30	38	117	0.8
-5	3 to 4	30	38	117	0.1
-20	3 to 4	30	38	117	0.1
-20 (Hydrated)	3 to 4	30	38	117	0.1