Differential Side Chain Hydration in a Linear Peptide Containing a Type VI Turn

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A high population of a compact type VI turn structure has been found in the *cis* isomeric form of five- and six-residue peptides of the general sequence AA-Ar-*cis*-Pro-Ar-Asp-(AA), where AA is any amino acid and Ar is an aromatic residue (Tyr, Phe, or Trp).[1,2] Stabilization of the turn structure is apparently provided by a close stacking interaction between the proline ring and the flanking aromatic rings, together with a secondary electrostatic interaction between the positively-charged N-terminus and the negative charges of the side chain of the aspartate at position 5 of the peptide.[2] Structures calculated for the folded form of the peptide NH_3^+-Ser-Tyr-Pro-Phe-Asp-Val-COO^- (SYPFDV)[3] clearly show a highly specific interaction between the three ring systems, as illustrated in Figure 1. In this paper we describe the results of an investigation of the hydration of the peptide NH_3^+-Ser-Tyr-Pro-Tyr-Asp-COO^- (SYPYD) which shows NMR behavior exactly analogous to that of the peptide SYPFDV, for which the solution structure was calculated.[3] Preliminary investigations with SYPFDV indicated that differential solvation of the proline protons with highly upfield-shifted resonances was present, but the results were equivocal due to interference from the overlapping resonances of the $C^{\gamma}H_3$ protons of valine. We therefore chose to use the similar peptide SYPYD, which contains an even higher population of type VI turn than SYPFDV, over 80% of the *cis* isomer according to several NMR criteria.[2] The present paper demonstrates that the formation of the type VI turn in the *cis* isomeric form of the peptide is accompanied by differential hydration of the proline ring protons that approach most closely the aromatic rings. These studies provide independent evidence for the compact and highly populated type VI turn structure and confirm that the stabilization of the turn occurs mainly as a result of highly specific hydrophobic interactions between the aromatic and proline rings.

One approach to the understanding of protein folding is based on the studies of propensities of peptide fragments of proteins to adopt native-like structure.[4] Early events in the folding of proteins, which are difficult to detect in intact proteins because of the rapid and highly cooperative nature of the folding process, can be modeled using short peptide fragments of proteins, since the long-range interactions that drive the folding process are necessarily absent. In recent years, a number of peptide fragments of proteins have been investigated by NMR and circular dichroism and shown to have conformational preferences for secondary structure.[5] Although it is clear from sequence, pH, and temperature dependence studies (for example, see refs 1 and 2) that intrapeptide interactions are important in stabilizing ordered structure, the role of the water solvent in these systems is not well understood. Theoretical studies using molecular dynamics simulations with explicit inclusion of water

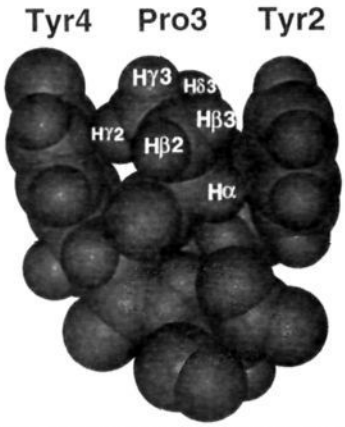

Figure 1. Space-filling representation of SYPYD adapted from one of a family of structures calculated for the type VI turn conformation of the *cis* isomeric form of the peptide SYPFDV[3] showing the close packing of the two aromatic rings and the proline ring.

molecules (reviewed in ref 6) indicate that water molecules tend to compete with intrapeptide hydrogen bonds and destabilize secondary structure.

NMR provides a powerful method for investigating the interaction of water with biological macromolecules via NOE- and ROE-type dipolar cross-relaxation processes between water protons and polypeptide protons.[7−10] This method has been applied to a number of proteins, including the globular protein BPTI[10] and the small, highly flexible "random coil" peptide oxytocin.[10] The behavior of BPTI should be representative for a globular protein: NOEs are seen from the protein to slowly exchanging interior water molecules (residence time $\tau > 10$ ns) and to a small number of fast exchanging surface water molecules (correlation time $\tau < 1$ ns). The major part of the interior of the protein does not come into contact with either bulk or bound water. By contrast, oxytocin is hydrated over its entire molecular surface, probably due to its small size and lack of highly ordered structure.[10] Similar studies reported herein for a peptide that is highly ordered in solution show that the method can give useful information in systems in conformational equilibrium, provided that the population of the folded conformer is sufficiently high.

Peptides were synthesized as described previously.[2] NMR samples of SYPYD (40 mM) were prepared in 90% ^1H_2O/10% ^2H_2O at pH 4.1 and 1.8. NMR spectra were obtained on a Bruker AMX 600 spectrometer, and the probe temperature was calibrated with methanol according to the method of Van Geet.[11] 2D NOESY and ROESY experiments were collected using a slightly modified version of the pulse sequences proposed by Otting et al.,[9] with the inclusion of a CW spin lock during the mixing time of the ROESY instead of a series of $\pi/6$ pulses. Typical parameters are $d = 1.8-2.0$ s for the recycle delay and $\delta = 160\ \mu$s for the defocusing delay. The mixing time τ_m was set to 250 ms for both the NOESY and ROESY spectra. The spin lock pulses were $SL_1 = 2$ ms with $\gamma B_1 \approx 10$ kHz for 2D ROESY spectra and $SL_1 = 1$ ms with $\gamma B_1 \approx 8$ kHz and $SL_2 = 2$ ms with $\gamma B_1 \approx 10$ kHz for 2D NOESY spectra. The rf-field strength for the ROESY mixing period was $\gamma B_1 \approx 5$ kHz. Quadrature detection in ω_1 was achieved using TPPI[12] in all

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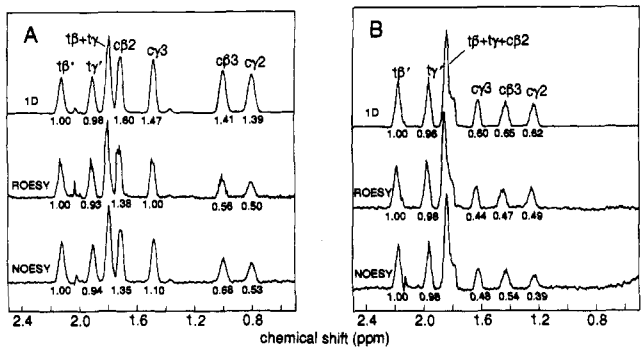

Figure 2. (A) Top: Upfield region of the one-dimensional NMR spectrum of 40 mM SYPYD in 90% ^1H_2O/10% ^2H_2O, pH 4.1, 278 K, showing the resonances of the proline C$^\beta$H and C$^\gamma$H protons in the *cis* (c) and *trans* (t) isomeric forms of the peptide. The ratio of *cis*:*trans* isomer is 1.4:1, obtained from the integrated intensities of the resonances. The resonances of the *cis* proline have been stereospecifically assigned as shown.[3] Middle and bottom: The same region of 1D traces of the 2D ROESY (middle) and 2D NOESY (bottom) spectra taken along ω_2 at the resonance frequency of H_2O in ω_1; these spectra were multiplied by -1. The integrated intensities of the resonances are shown below each peak, normalized to the area of the resonance of the *trans* C$^\beta$H at 2.1 ppm. The integrated intensity of the overlapped peak labeled as tβ + tγ is 2.02 (top), 1.87 (middle), and 1.84 (bottom) on the same scale. All spectra were corrected for the spectral excitation profile sin[0.603($\delta-\delta_w$)], where δ_w is the chemical shift of water in the middle of the spectrum. (B) Identical experiment as in A at pH 1.8 and 303 K. The ratio of *cis*:*trans* isomer under these conditions is 0.6:1 based on integrated intensities of the resonances. The integrated intensity of the overlapped peak labeled as tβ + tγ + cβ2 is 2.62 (top), 2.63 (middle), and 2.63 (bottom).

pulse sequences, with the carrier placed at the water resonance. The first t_1 delay was set to $\Delta t = 4\tau_{90}/\pi$, where τ_{90} is the length of the 90° pulse, to avoid frequency-dependent phase corrections.[13] During processing the FIDs were shifted to the right by one point and the first point was set to zero or linear back-predicted; 512 t_1 data points with 64 scans each were collected. Some of the early spectra showed the effects of radiation damping, manifested as dispersive components in cross sections along ω_2 through the water resonance. These effects were eliminated by the use of homospoil pulses during the mixing time of the NOESY experiment. Data were processed using an Iris Indigo 4D computer with FELIX software. Processed spectra were typically stored in 2K × 2K real matrices. The residual water signal was removed from the spectra by application of a sine bell low-pass filter on the time domain data,[14] and 90°-shifted sine bell window functions were used along t_1 and t_2. Linear or higher order polynomial base-line correction was applied after Fourier transformation along the acquisition dimension.

Figure 2 shows a comparison of the one-dimensional spectrum of SYPYD together with cross sections at the water frequency in ω_1 in the NOESY and ROESY spectra under two different sets of conditions. All of the water cross peaks in the NOESY and ROESY spectra originate from positive cross-relaxation rates; i.e., they have opposite sign to the diagonal, and they reflect short water correlation times (τ < 1 ns). The integrated peak intensity of the resonances indicates that the two forms of the peptide, *cis* and *trans*, are present in different proportions in the two experiments, 1.4:1 (60% *cis*) at pH 4.1, 5 °C (Figure 2A), and 0.6:1 (38% *cis*) at pH 1.8, 30 °C (Figure 2B). This is consistent with a lowered population of the type VI turn conformation at the lower pH and higher temperature;[2] the spectra obtained under these conditions therefore act as a control. Estimates of the population of the type VI turn conformation in the *cis* form of SYPYD exceed 80% at pH > 5 and low temperature compared to <*ca* 25% at pH 1.8, 30 °C. The resonances of the proline ring protons in the *cis* form of the peptide are significantly upfield-shifted, consistent with the effect of the ring currents of the two tyrosine rings, which pack tightly and specifically onto the proline ring in the type VI turn conformation[3] (Figure 1). The extent of the upfield shift gives another measure of the population of the folded form;[2] by this criterion, the population of the folded form in the control experiment is substantially reduced but not eliminated.

The three spectra shown in Figure 2 for each set of conditions (cross sections at the water resonance frequency of the NOESY and ROESY spectra, together with a 1D spectrum of SYPYD for comparison) have been normalized with respect to the integrated intensity of the Pro C$^\beta$H resonance (2.1 ppm) of the *trans* form of the peptide. For the data in Figure 2A it is clear that the intensities of the NOE and ROE cross peaks from the water proton resonance to the proline C$^\beta$H and C$^\gamma$H resonances in the *cis* form of the peptide are reduced. The NOEs and ROEs from water to the C$^{\beta3}$H and C$^{\gamma2}$H resonances are affected to the greatest extent; their intensities vary between 36% and 48% of those of the corresponding resonances in the 1D spectrum. The C$^{\beta2}$H and C$^{\gamma3}$H resonances of the *cis* proline are affected to a lesser extent, with relative intensities ranging between 68% and 86%. Thus, the proline C$^{\beta3}$H and C$^{\gamma2}$H protons are less exposed to bulk water than the C$^{\beta2}$H and C$^{\gamma3}$H protons. By contrast, at pH 1.8 and 303 K (Figure 2B) no difference is observed in the relative intensities of the NOEs between the water protons and the *cis* proline C$^{\beta3}$H, C$^{\gamma2}$H, or C$^{\gamma3}$H resonances. Under both conditions NOESY and ROESY cross sections show similar intensities, which is characteristic for short water–peptide correlation times (τ < 100 ps),[10,15] consistent with cross relaxation with bulk water. Interference arising from exchange of the tyrosine hydroxyl protons with water can be ruled out for the proline ring protons. Under the conditions of Figure 2A, pH 4.1 and 278 K, intrapeptide NOEs are negative, causing exchange-mediated NOEs to water to become smaller than the corresponding ROEs; indeed, such effects are observed for the serine C$^\beta$H and tyrosine C$^\epsilon$H resonances. Since the NOE and ROE are of approximately equal intensity for the proline C$^\beta$H and C$^\gamma$H protons, contributions from tyrosine hydroxyl proton exchange mechanisms must be insignificant. Differential solvation of *cis* proline C$^\beta$H and C$^\gamma$H protons is also observed for SYPFDV, in which Tyr 4 is replaced by Phe, lending further support to our interpretation.

Our results indicate that certain of the proline ring protons are sterically hindered from interactions with the water solvent at pH 4.1 and 278 K. In the control experiments carried out under conditions (pH 1.8, 30 °C) where the type VI turn population is greatly reduced, the *cis* proline resonances are still slightly more shielded from bulk water than their *trans* counterparts but the differential NOE intensities between C$^{\beta2}$H and C$^{\beta3}$H and between C$^{\gamma2}$H and C$^{\gamma3}$H, respectively, in the *cis* peptide are not observed due to the lower population (<*ca* 25%) of the turn. We therefore conclude that the folded type VI turn conformation, with the close, specific packing between the proline and aromatic rings shown in the calculated structures (Figure 1), is the cause of the observed reduction in water accessibility to the proline ring protons. In contrast, unfolded peptides generally show uniform NOEs to all protons from water.[10] The differential hydration observed for SYPYD provides direct evidence for the stabilization of the turn structure by hydrophobic interactions between the aromatic and proline rings.

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