Effects of H$_2$O and D$_2$O on Polyproline II Helical Structure

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In this work we show that D$_2$O stabilizes the left-handed polyproline II (P$_{II}$) helical conformation of peptides relative to H$_2$O. The interaction of solvent with a polypeptide chain is one of the primary factors controlling protein folding and stability. In biologically relevant systems, this solvent is most often water. Estimates of the effects of water on peptide folding can be obtained from solvent perturbation experiments. The simplest perturbant for H$_2$O water is its isotopic D$_2$O form. D$_2$O has a greater average number of hydrogen bonds per molecule and a larger entropic cost for solvating molecules than H$_2$O.[1] D$_2$O decreases the enthalpy of unfolding in proteins, though stability is largely unchanged.[2] The solvation of peptides known to form P$_{II}$ helices with D$_2$O increases their propensity to adopt the P$_{II}$ conformation relative to H$_2$O as the solvent.

The P$_{II}$ structure is a left-handed 3$_1$ helix with ideal backbone dihedral angles around $\phi = -75°$ and $\psi = +145°$.[3] It has been shown using NMR and CD spectroscopy that a seven-residue alanine peptide adopts the P$_{II}$ conformation in an aqueous environment.[4] Other work has shown that alanine possesses significant P$_{II}$ character in situations that preclude α-helix formation.[5-7] Under such conditions, it is believed that backbone solvation favors P$_{II}$ helix formation.[4,5,8,9] The side chain of alanine is thought to be small enough not to interfere with solvation. The mechanism of backbone solvation is not fully understood but is believed to be more complex than just solvent accessibility.[8,10-13]

Interactions between solvent and the peptide backbone are believed to play a major role in non-proline residues adopting the P$_{II}$ conformation.[4,6,8,14,15] Pappu and co-workers have hypothesized that the P$_{II}$ conformation arises as a result of minimization of intrapeptide steric conflicts and favorable interactions with solvent that compensate for attractive intrapeptide interactions.[11] Kentsis et al.[12] and Mezei et al.[13] recently reached similar conclusions. Others have suggested that bridging water molecules are responsible for an alanine dipeptide adopting the P$_{II}$ conformation.[10]

These models are tested using a host−guest system consisting of peptides of sequence acetyl-(Pro)$_3$-X-(Pro)$_3$-Gly-Tyr-NH$_2$, where X is one (A), three (A3), five (A5), or seven (A7) alanines or one (V) or three (V3) valines. The N- and C-termini were acetylated and aminated, respectively, to remove strong electrostatic interactions, and the C-terminal tyrosine was included for concentration determination.[16] The proline-based host system aids with solubility.[7,9] It should be noted that a residue followed by a proline residue is restricted to the β-region of (ϕ,ψ) space due to steric constraints.[17] Since it is energetically more costly to disrupt the bulk structure of D$_2$O than H$_2$O, examination of these peptides in both solvents reveals the relationship between disruption of solvent structure and propensity to adopt the P$_{II}$ conformation. Peptides were obtained from Peptidogenic Research and Co., purified, and examined using circular dichroism (CD) as described in previous work.[9]

CD spectra of peptides containing seven alanines or a single valine guest residue, in D$_2$O and H$_2$O, are shown in Figure 1. The
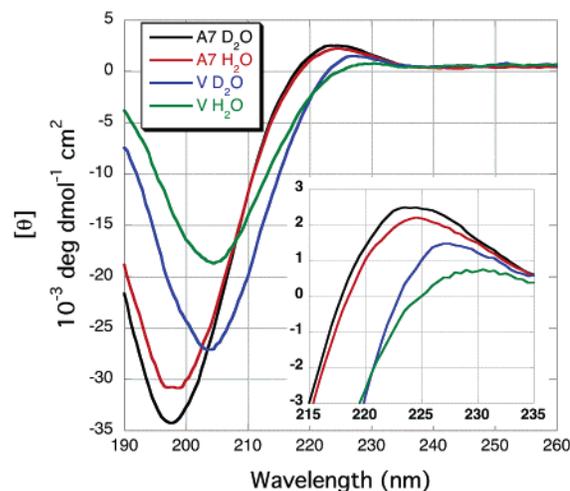

Figure 1. CD spectra of peptides in D$_2$O and H$_2$O, collected at 5 °C.

spectrum for each peptide clearly indicates the presence of the P$_{II}$ conformation. These spectra possess positive bands around 224−228 nm and negative bands around 197−205 nm, hallmarks of the P$_{II}$ helical conformation. The P$_{II}$ helix is the only secondary structure with a positive band in this region,[18] so we use this as a measure of the conformation. The P$_{II}$ CD bands move to lower wavelengths as more non-proline residues are added to the host peptide, a result of the different absorbance properties of primary, secondary, and tertiary amides.[18] The bands can also shift to higher or lower wavelengths due to contributions from other secondary structures. This is likely the reason for the shifts between identical peptides in D$_2$O and H$_2$O seen in Figure 1.

Due to steric effects of prolyl rings, the first, second, fourth, and fifth prolines in each peptide are restricted to the P$_{II}$ region of (ϕ,ψ) space.[19] As a result, CD spectra presented here possess a base P$_{II}$ content that is independent of the effects of guest residues. The heights of the positive bands in the CD spectra decrease as the number of alanine guest residues is increased, indicating that the P$_{II}$ content decreases (Figure 2). Previous work demonstrated that valine guest residues strongly disfavor the P$_{II}$ conformation, so the P$_{II}$-like CD signals for V and V3 in H$_2$O in Figure 2 are primarily due to the host peptide prolines.[6,7]

On the basis of the results of the solvent−P$_{II}$ interaction work of Pappu and co-workers,[11] we hypothesize that D$_2$O would stabilize P$_{II}$ helices relative to H$_2$O. An interpretation of the results of Pappu and co-workers is that the P$_{II}$ conformation is favored by solvent, in part because it disrupts bulk water less than other secondary structures.[11] D$_2$O is known to be more ordered than H$_2$O with a greater energy requirement for solvating solutes,[1] so minimizing the disruption of bulk water structure would have a greater effect in D$_2$O.

The heights of the positive bands in Figure 2 are consistently higher for peptides in D$_2$O versus those in H$_2$O, indicating a
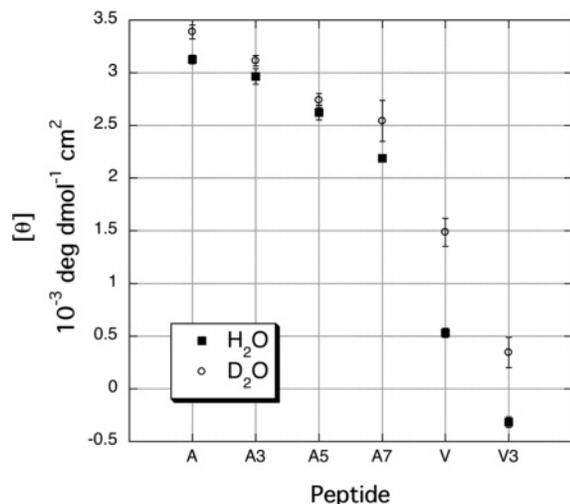
Figure 2. Heights of the positive bands from CD spectra collected at 5 °C in H_2O and D_2O for peptides examined in this work.
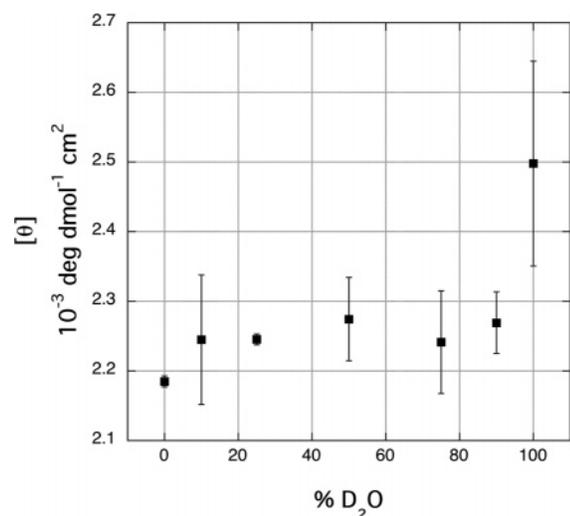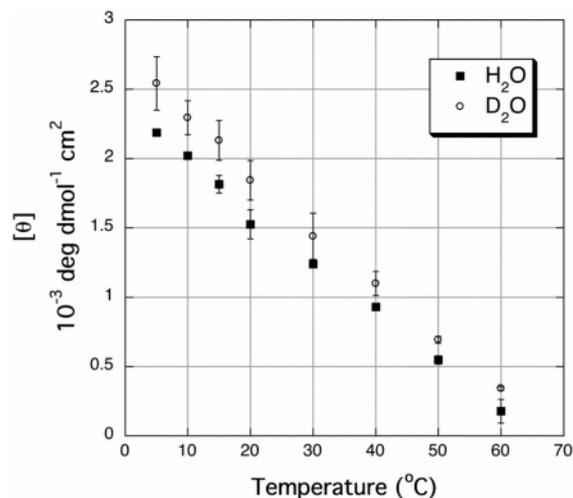

Figure 3. Height of the positive band in the CD spectra of A7 at 5 °C as a function of D_2O concentration.

consistently higher P_{II} content. This predilection for P_{II} helix grows in a nonlinear fashion with D_2O content, as indicated in Figure 3. This is consistent with the nonlinear behavior of the properties of H_2O/D_2O mixtures.[20] The differences between H_2O and D_2O vary by peptide, indicating a sequence dependence, and are larger than error. Alanine has an intrinsically high P_{II}-forming propensity,[4-7,9] which explains the small difference between the P_{II} content of the peptide in H_2O and D_2O. Valine has a low propensity to adopt the P_{II} conformation,[6,7,9] allowing for a larger D_2O effect on peptide structure.

Figure 4 shows the P_{II} content, as indicated by the height of the positive CD band, of the A7 peptide decreases linearly with temperature in both D_2O and H_2O. At all temperatures the peptide has a higher P_{II} content in D_2O. The difference in A7 P_{II} content in D_2O and H_2O decreases with increasing temperature, indicating a convergence of the structuring properties of the two solvents, although the basis for this is not clear.

Experiments demonstrating a difference in peptide conformation in H_2O and D_2O are of great importance. Researchers exploring

Figure 4. Heights of the positive bands in the CD spectra of A7 in D_2O and H_2O as a function of temperature.

peptide and protein structure using NMR, VCD, IR, and Raman spectroscopy use samples solvated in part or in whole by D_2O. D_2O favors P_{II} structure, so the results of these experiments will have a bias toward that conformation. Although the bias is small, it is significant, particularly in the case of valine, which has one of the lowest propensities to adopt the P_{II} conformation. The P_{II} structuring effect of D_2O supports the hypothesis of Drozdov et al.[11] and the computations of Kentsis et al.[12] and Mezei et al.[13]

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