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Water-Induced Switching of β-Structure to Polyproline II Conformation in the 4*S*-Aminoproline Polypeptide via H-Bond Rearrangement

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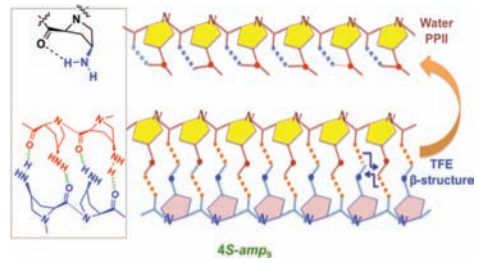
ABSTRACT



4*S*-Aminoproline polypeptide 2 forms unusual β-structure in trifluoroethanol that switches to the polyproline II (PPII) form in aqueous medium, while 4*R*-aminoproline peptide 1 retains PPII form in both solvents. This first instance of a polyproline derivative showing a β-structure is attributed to competitive pH-dependent (4-NH_3^+/NH_2) stereoelectronic effect (4*R* vs 4*S*) and the overriding importance of stereospecific intra/intermolecular H-bonding in (2,4)-*cis*-4*S*-aminoproline in contrast to (2,4)-*trans*-4*R*-aminoproline oligomers.

The polyproline type II (PPII) helix is a prevalent conformation in both folded and unfolded proteins[1] and plays an important role in a wide variety of biological processes, such as signal transduction, transcription, immune response, and cell motility.[2] Each strand of collagen triplex with the Pro-Hyp-Gly tripeptide repeat unit adopts a left-handed PPII-like conformation.[3] Oligoprolines and their derivatives have found utility as cell penetrating agents[4] and as molecular spacers in biomimetic systems for energy/electron transport.[5] The PPII helix is a fully extended left-handed structure with all amide bonds in the *trans* conformation, while the right-handed PPI helix is compact, with all amide bonds in the *cis* conformation.[1] It is well-known that polyprolines adopt PPII conformation in water[6] and PPI conformation in hydrophobic solvents of short chain aliphatic alcohols.[6] It has been well demonstrated that the stereoelectronic effect

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of the 4-substituent plays a major role in determining the pucker of the pyrrolidine ring of proline and hence the conformational stability of proteins.[7]

In this context, we reported earlier that 4-amino substitution on proline in collagen peptide stabilizes the triple helix[8] at both acidic and basic pHs. In collagen, the tripeptide repeat unit [Pro-Hyp-Gly] has glycine whose amide linkage is involved in an interchain H-bond, leading to the triple helix structure. In contrast, polyproline peptides lack amide NH and hence are unable to form a triplex via interchain H-bonds, ending up as a single helix of PPI or PPII type. Recently, 4*S*(OH/NH_3^+) groups on proline were shown to form intramolecular H-bonds with the amide carbonyl, increasing the *trans*/*cis* amide ratio and thereby promoting PPII conformation in the derived polypeptides.[9] Unlike other 4-substituents on proline studied so far (OH, SH, CH_3, F), the ionizable 4-NH_2 group is a good probe to examine the pH effects on polyproline conformation. Herein, we report the novel behavior of 4*S*-aminoproline polypeptide to form a novel β-structure in trifluoroethanol (TFE) that switches to PPII form in aqueous medium. This property exclusive to 4*S*-aminoproline polypeptide arises from a stereospecific *intra*molecular H-bonding that stabilizes the PPII form, while the unusual β-structure results from *inter*chain H-bonding. To our knowledge, this is perhaps the first report of formation of β-structure in any polyproline derivatives and its switch over to PPII form induced by water.

Synthesis and Conformational Studies of 4-Aminoproline Oligomers. The oligopeptides **1**−**3** were synthesized from appropriate N-Fmoc-protected monomers asssembled in the solid phase, purified by HPLC, and characterized by MALDI-TOF (for details, see Supporting Information). The CD spectral analyses were carried out as a function of temperature, pH, urea, and solvents (buffer and trifluoroethanol, TFE). All three peptides (100 μM, pH 7.2) show CD spectra (Figure 1) with a positive band between 220 and 230 nm and a negative band between 200 and 210 nm that are the established patterns of the PPII conformation.[10] The intensity of the positive band at 225−227 nm is proportional to the PPII helical content which is seen to decrease in the order 4*R*-*Amp*$_9$ **1** > 4*S*-*amp*$_9$ **2** > Pro$_9$ **3**.

The effect of protonation of the 4-amino group on PPII helical content was examined by the CD spectra of peptides **1**−**3** (Figure 2A) recorded at different pH (4.0−10.0). The positive ellipticity at 225 nm for 4*R*-*Amp*$_9$ **1** decreased by 10% with increasing pH up to 7.2 and did not change further until pH 10.0. In the case of 4*S*-*amp*$_9$ **2**, positive intensity was enhanced in the pH range 4.0−10.0 in a sigmoidal

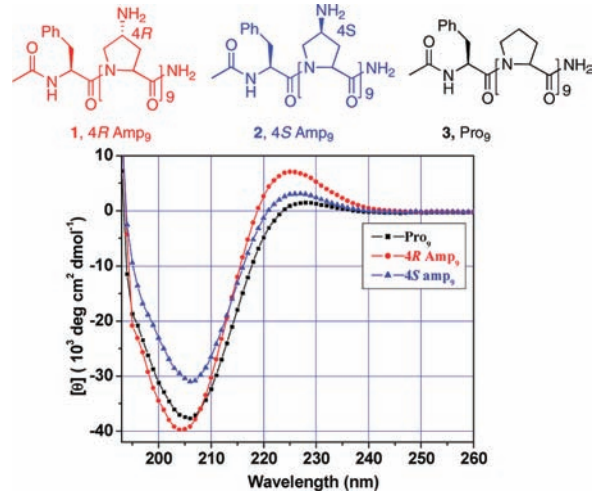

Figure 1. CD profiles of polypeptides **1**, 4*R*-*Amp* (●); **2**, 4*S*-*amp* (▲); and **3**, *Pro*$_9$ (■), all at 100 μM (pH 7.2).

fashion. At acidic pH (4.0−5.0), the PPII helicity of 4*S*-*amp*$_9$ **2** was low (20% of 4*R*-*Amp*$_9$ **1**) but increased by 2-fold at pH 10.0. The ellipticity of peptide *Pro*$_9$ **3** remained

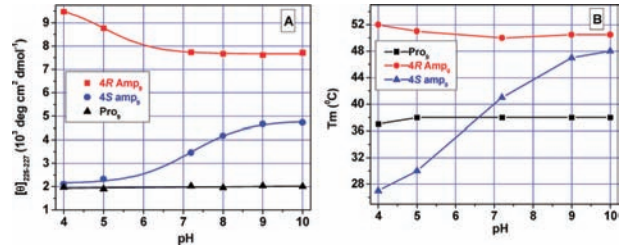

Figure 2. (A) Intensity of the positive band of CD spectra of peptides **1**−**3** as a function of pH. (B) Thermal stability of PPII helices in peptides **1**−**3** as a function of pH, followed at 225 nm.

constant with pH. This suggests a pivotal role for both stereochemistry and the protonation status of the 4-amino group in eliciting the PPII helicity of 4(*R*/*S*)-aminoproline polypeptides **1** and **2**.

The pH-dependent thermal stability (T_m) of PPII helices in peptides **1**−**3** was measured from temperature-dependent CD spectral data (Figure 2B and Supporting Information). It is seen that (i) 4*R*-*Amp*$_9$ **1** has maximum T_m at all pHs; almost invariant (ii) 4*S*-*amp*$_9$ **2** has the lowest T_m among the peptides at pH 4.0 but increased gradually with raise in pH to 10.0 to a value closer to the T_m of 4*R*-*Amp*$_9$ **1**; and (iii) *Pro*$_9$ **3** with intermediate T_m at pH 4.0 remained constant over the pH range. The 4-NH_3^+ group at pH 4.0 stabilized the PPII helix most in the 4*R*-form and least in the 4*S*-form, while 4-NH_2 at pH 10.0 stabilized both 4*R*- and 4*S*-peptides to a similar extent. The 4*S*-*amp*$_9$ **2** thus exhibited significant pH-dependent PPII stability that is maximum in the unionized amino form.

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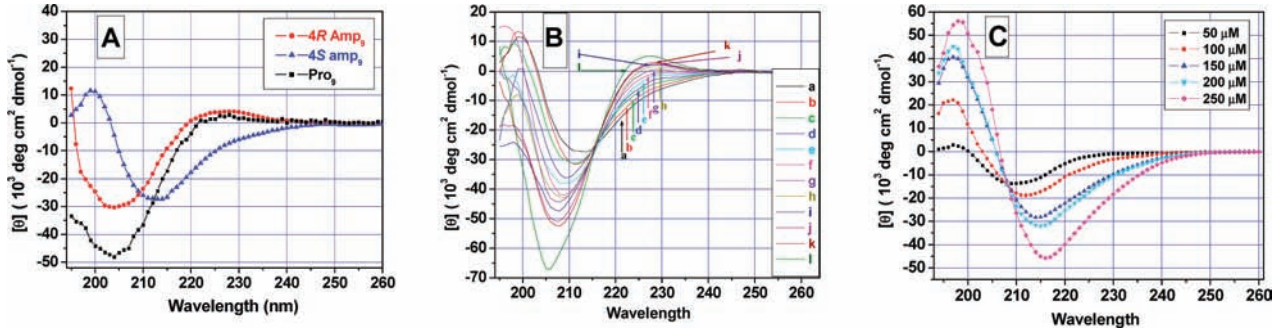


Figure 3. (A) CD spectra of peptides **1**−**3** in trifluoroethanol (TFE). (B) CD spectra of 4*S*-*amp*$_9$ **2** in TFE with incremental addition of phosphate buffer (pH 7.2) from (a) 0.1% to (k) 1.0% in 0.1% steps and (l) 2.0%. (C) Increasing concentration of **2** from 50 to 250 μM.

Urea is known to enhance the PPII helical content through rigidification of the polypeptide backbone.[11] The low PPII helicity of 4*S*-*amp*$_9$ **2** was enhanced enormously (>300%) by addition of 1 M urea (pH 7.2), while that of 4*R*-*Amp*$_9$ **1** and (*Pro*$_9$) **3** increased by a mere 15−20% (Supporting Information). The larger changes seen specifically for 4*S*-*amp*$_9$ **2** in the presence of urea and upon increasing the pH suggest the combined role of H-bonding and stereoelectronic[12] effects in dictating the PPII conformation.

Solvent plays a key role in modulating the H-bonding effects, and hence the CD spectra of peptides **1**−**3** were recorded in a flourinated solvent trifluoroethanol (TFE) (Figure 3A). The 4*R*-*Amp*$_9$ **1** and *Pro*$_9$ **3** show CD spectra typical of PPII form. Very interestingly, the CD spectra of 4*S*-*amp*$_9$ **2** in TFE are unlike the PPII pattern, showing a negative maximum around 214 nm and a broad shoulder at 228 nm that is typical of β-structure.[13]

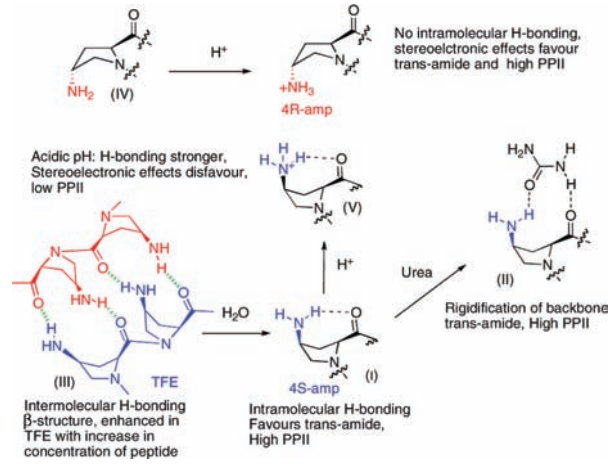
When aqueous phosphate buffer (pH 7.2) was titrated into TFE solution of 4*S*-peptide **2** in tiny incremental steps of 0.1%, the 214 nm negative band slowly shifted to 205 nm, accompanied by a growing of the broad negative shoulder at 228 nm into a positive band at about 224 nm (Figure 3B) typical of PPII form. The isosbestic point seen at 215 nm is indicative of the conversion of 4*S*-peptide **2** from β-structure in 100% TFE to full PPII form with 0.8% buffer in TFE.

Upon increasing the concentration of 4*S*-*amp*$_9$ **2** from 50 to 250 μM in TFE, the CD spectra exhibited a nice growth in the negative band intensity at 210 nm, accompanied by its shift to 216 nm and a large increase of the positive band at 200 nm (Figure 3C). As expected for *inter*molecular hydrogen bonding increasing with concentration, this strongly points to a consolidation of β-structure in the peptide 4*S*-*amp*$_9$ **2**. In the case of 4*R*-*Amp*$_9$ **1**, increasing the peptide concentration leads to enhancement of the PPII form without any other changes. The overall results imply that 4*S*-*amp*$_9$ **2** assumes a β-structure in TFE that is transformed to the PPII form in aqueous medium, unlike 4*R*-*amp*$_9$ **1** which retains the PPII form in both conditions.

The formation of β-structure in polyproline peptides under any conditions is unprecedented in the literature since they lack H-bond donor sites. In 4*S*-*amp*$_9$ **2**, the NH_2 group can form an *intra*molecular H-bond with the amide carbonyl of the same proline moiety, promoting a PPII conformation.[9b] The possibility of the 4*S*-NH_2 group engaging the amide carbonyl of another chain of 4*S*-*amp*$_9$ through an *inter*molecular H-bond would lead to β-structure. Such an *inter*chain H-bonded structure should be facilitated at higher peptide concentration.

A plausible molecular picture for this conversion is depicted in Scheme 1. The *intra*molecular H-bonding of 4*S*-

Scheme 1. Solvent-Derived Rearrangement of H-Bonds in 4*S*-*amp*$_6$ Leading to Conformational Switch



NH_2 with amide carbonyl possible only in 4*S*-*amp*$_9$ **2** (I) promotes the PPII form in buffer. Urea rigidifies the backbone[11] by complementary H-bonding (II) among the *cis*-disposed 4*S*-NH_2 group and the amide carbonyl to strengthen the PPII form. In a fluorinated solvent TFE, the intramolecular H-bonding between 4*S*-NH_2 and the amide carbonyl switches to interchain H-bonding (III) giving rise to antiparallel β-structure. The *trans* disposition of 4*R*-NH_2 and

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the amide carbonyl group in 4*R*-*Amp*$_9$ (IV) is not conducive to formation of either intramolecular or strong interchain H-bonding in the derived peptide.

Stereoelectronic vs H-Bonding Effects in 4*S*-*amp*$_9$ 2. The stereoelectronic effect of 4*R*-X substituents on proline is known to strongly favor the PPII form, over that of 4*S*-X substituents by enhancing the *trans*-amide content.[12] Although the intramolecular H-bonding in 4*S*-NH_3^+ favors the *trans*-amide form,[9b] the unfavorable stereoelectronic effect of 4*S*-NH_3^+ (unlike 4*R*-NH_3^+) strongly negates the benefit of H-bonding (V), leading to a low PPII form for **2** at acidic pH. At higher pH, the effect of intramolecular H-bonding dominates a weaker stereoelectronic effect of 4*S*-NH_2, promoting higher PPII content in peptide **2**. While the conversion of PPII to PPI takes many hours/days due to a slow conversion of amide from *trans* to *cis* form,[14] the switching of β-structure to PPII form is fast, within minutes, suggesting that the amide bond of **2** is also in *trans* form in the β-structure. No PPI form was seen for any of the peptides **1**−**3**, under different conditions of pH, *n*-propanol and TFE.

In conclusion, it is demonstrated here that 4*S*-*amp*$_9$ **2** adapts an unusual β-structure in TFE unlike most polyproline peptides which prefer the PPI form in hydrophobic/fluorinated media. The β-structure arises from *inter*chain hydrogen bonds involving 4*S*-NH_2 and amide carbonyl, which are broken in water and rearranged to *intra*molecular H-bonding that favors the PPII form via enriching the *trans*-amide geometry. This structural conversion illustrates a fine balance between stereoelectronic and H-bonding effects in novel tuning of the secondary structure of 4*R*/*S*-aminoproline polypeptides. β-Structure in polyproline peptides is hitherto unknown, and the present results will add a new design principle to a growing repertoire of strategies for engineering peptide secondary structural motifs for new biomaterials and nanoassemblies.[15]

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Supporting Information Available: Experimental synthesis procedures, HPLC, mass spectral data, and CD spectra of peptides **1**−**3** under different conditions of pH, urea, salt, and concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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