Peptide Revolution: Genomics, Proteomics & Therapeutics
Michael Chorev & Tomi K. Sawyer (Editors)
American Peptide Society, 2003

Effect of 4-Fluoroproline in the X-Position on the Stability of the Collagen Triple Helix

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Introduction

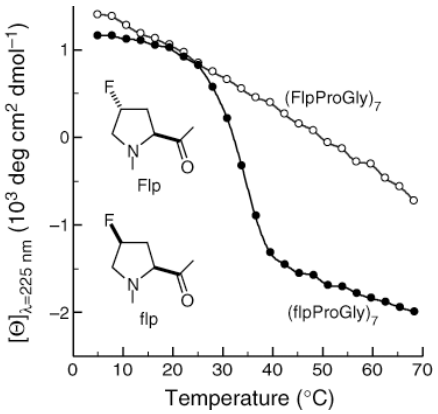
Collagen consists of three polypeptide chains that fold into a triple helix. Each natural chain contains many repeats of the sequence: XaaYaaGly, in which a third of the Xaa and Yaa residues are (2*S*)-proline (Pro). The pucker of a proline ring can be influenced by electronegative substituents, such as the hydroxyl in the naturally occurring residue, (2*S*,4*R*)-4-hydroxyproline (Hyp) [1,2]. This effect is stereoelectronic, as it depends on the configuration and electron-withdrawing ability of the substituent. In particular, the *gauche* effect exerted by an electron-withdrawing 4*R* substituent stabilizes the C^{γ}-exo pucker, and that by a 4*S* substituent stabilizes the C^{γ}-endo pucker. The degree of stabilization is likely to be greatest for fluorine, the most electronegative of atoms.

The thermal stability of the triple helix is increased by replacement of proline in the Yaa position with Hyp [3] and, to a greater degree, (2*S*,4*R*)-4-fluoroproline (Flp) [4]. Molecular modeling of a triple helix of $(ProProGly)_{10}$ strands has suggested that Pro in the Xaa position prefers to adopt a C^{γ}-endo pucker, whereas Pro in the Yaa position prefers a C^{γ}-exo pucker [5]. This pattern has been observed in a crystalline $(ProProGly)_{10}$ triple helix [6]. The pyrrolidine ring pucker influences the range and distribution of the ϕ and ψ main-chain dihedral angles of Pro, and can fix those dihedral angles for optimal packing of the triple helix. Increasing the preference for the desired C^{γ}-exo conformation in the Yaa position by inclusion of either Hyp or Flp decreases the entropic penalty for triple-helix formation. Likewise, Hyp and Flp increase the preference of the ω main-chain dihedral angle for the *trans* ($\omega = 180°$) conformation [7]. Because all peptide bonds in collagen are *trans*, preorganization of ω by Hyp and Flp decreases the entropic penalty for triple-helix formation.

Results and Discussions

Can triple-helix stability be increased by fixing the ring pucker of proline in the Xaa position? We synthesized peptides with both diastereomers of 4-fluroproline in the Xaa position [8]. Circular dichroism spectroscopy indicates that only $(flpProGly)_7$, where flp refers to (2*S*,4*S*)-4-fluoroproline, forms a stable triple helix at 5 °C. During thermal denaturation, $(flpProGly)_7$ exhibits a cooperative transition characteristic of a triple helix (Figure 1). The midpoint of this transition is at 33 °C. The linear decrease in elipticity by $(FlpProGly)_7$ is characteristic of the unfolding of a single polypeptide chain. Sedimentation equilibrium experiments confirm that $(FlpProGly)_7$ but not $(flpProGly)_7$ is a monomer at 4 °C, whereas both peptides are monomers at 37 °C.

Apparently, stereoelectronic effects can operate adventitiously (or deleteriously) in the Xaa position of collagen. There, flp is able to preorganize the ϕ and ψ dihedrals as in a triple helix without encountering the steric conflicts that appear to plague (2*S*,4*S*)-4-hydroxyproline (hyp) in this position [6,9]. Moreover, the 4*S* substituent in the Xaa position has limited access to solvent, thus making fluoro better suited than hydroxyl to occupy this position. Altogether, the gain in stability upon replacing hyp with flp in the Xaa position exceeds that of replacing Hyp with Flp in the Yaa position (Figure 1).



	T_m (°C)	
Xaa/Yaa	$(XaaProGly)_7$	$(ProYaaGly)_7$
Flp	no helix [8]	45 [7]
Hyp	no helix [10]	36 [7]
Pro	6–7 [11]	6–7 [11]
Hyp	no helix [9]	no helix [9]
flp	33 [8]	no helix [7]

Fig. 1. (Left) Thermal denaturation curves determined by measuring molar elipticity at 225 nm as a function of temperature [8]. (Right) Effect of 4-hydroxyproline (Hyp and hyp) and 4-fluoroproline (Flp and flp) diastereomers on the conformational stability of a collagen triple helix with $(XaaYaaGly)_7$ strands. "No helix" refers to T_m < 5 °C. Results with Hyp in the Xaa position and hyp in both the Xaa and Yaa positions are for $(XaaYaaGly)_{10}$ strands.

Because the stability of $(flpProGly)_7$ exceeds that of $(FlpProGly)_7$, the preorganization of ϕ and ψ in the Xaa position is more important than is the preorganization of ω [8]. This constraint could be less important for proline-poor regions of the triple helix, in which a non-proline residue occupies the Xaa or Yaa position. The structure of a collagen mimic indicates that proline-rich and proline-poor regions have a distinct triple-helical twist [12], which suggests that the factors that control stability could differ for these regions. Indeed, replacement of proline in the Xaa position with Hyp does increase the stability of a proline-poor region [13].

Hence, the conformational stability of collagen can be enhanced by stereoelectronics effects. We anticipate that the rational use of stereoelectronic effects could be used to enhance the conformational stability of other proteins as well.

Acknowledgments

This work was supported by grant AR44276 (NIH). J.A.H. was supported by postdoctoral fellowship AR48057 (NIH).

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