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Stereoelectronic Effects on Collagen Stability: The Dichotomy of 4-Fluoroproline Diastereomers

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Stereoelectronic effects can impose constraints on the conformation of organic molecules.[1] Nonetheless, only one stereoelectronic effect on the conformation of a protein is known.[2] That unique effect is manifested in collagen, the most abundant protein in animals.

Collagen consists of three polypeptide chains that fold into a triple helix.[3] 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) or (2*S*,4*R*)-4-hydroxyproline (Hyp).[4] Replacing Pro or Hyp in the Yaa position with the nonnatural amino acid (2*S*,4*R*)-4-fluoroproline (Flp) greatly increases triple-helix stability.[5] In contrast, replacing Pro or Hyp in the Yaa position with the diastereomer (2*S*,4*S*)-4-fluoroproline (flp) greatly decreases stability.[2] Here, we use fluorine substitution to probe for stereoelectronic effects in the Xaa position of collagen. Again, we find that one diastereomer of 4-fluoroproline is greatly stabilizing to the triple helix, and the other is greatly destabilizing. Remarkably, the stereoelectronic preference of the Xaa position is opposite to that of the Yaa position, as flp but not Flp endows hyperstability.

The pucker of a pyrrolidine ring can be influenced by electronegative substituents.[5,6] This effect is stereoelectronic, as it depends on the configuration and electron-withdrawing ability of the substituent.[2,7] In particular, the gauche effect[6b,8] exerted by an electron-withdrawing 4*R* substituent stabilizes the C$^\gamma$-exo pucker, and that by a 4*S* substituent stabilizes the C$^\gamma$-endo pucker. The degree of stabilization is likely to be greatest for fluorine, the most electronegative of atoms.

Molecular modeling of a triple helix of $(ProProGly)_{10}$ strands has suggested that Pro in the Xaa position prefers to adopt a C$^\gamma$-endo pucker, whereas Pro in the Yaa position prefers a C$^\gamma$-exo pucker.[9] This pattern has been observed in a crystalline $(ProProGly)_{10}$ triple helix.[10] The pyrrolidine ring pucker influences the range and distribution of the ϕ and ψ main-chain dihedral angles[11] of Pro and can fix those dihedral angles for optimal packing of the triple helix. Increasing the preference for the desired C$^\gamma$-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[11] for the trans ($\omega = 180°$) conformation.[2,6b,7a] Because all of the peptide bonds in collagen are trans, preorganization of ω by Hyp and Flp decreases the entropic penalty for triple-helix formation.

As in the Yaa position, preorganization of ω in the trans conformation would also be favorable in the Xaa position. Yet, a C$^\gamma$-exo conformation favors ϕ and ψ dihedrals that are not ideal for this position. Hence, fixing the ring pucker of proline in the Xaa position could have a favorable influence on either ϕ, ψ, or ω, but not all three.

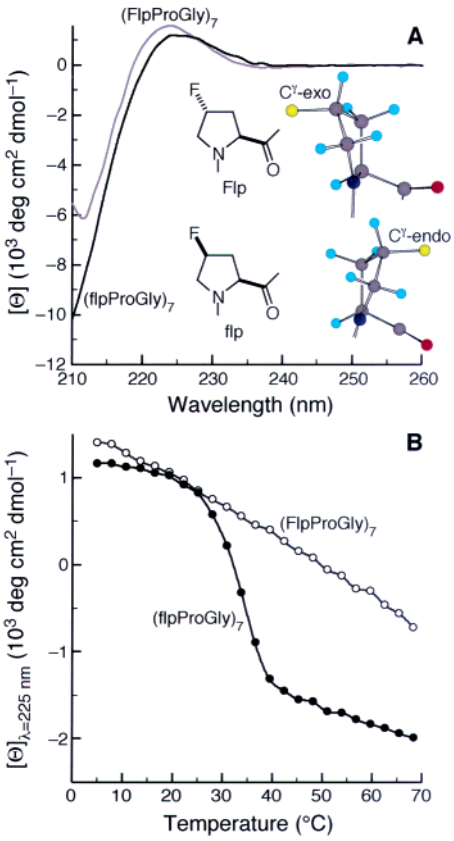

Figure 1. (A) Circular dichroism spectra at 5 °C. Solutions of peptide (0.2 mM in 50 mM acetic acid) were allowed to incubate at 4 °C for ≥24 h before spectra were recorded. Inset: Structures of Flp and flp from ref 7. (B) Thermal denaturation curves determined by measuring molar elipticity at 225 nm as a function of temperature (±1 °C). Data were recorded at intervals of 3 °C after equilibration for ≥5 min.

Can triple-helix stability stability be increased by fixing the ring pucker of proline in the Xaa position? Replacing Pro in the Xaa position of $(ProProGly)_{10}$ with either Hyp[12] or its diastereoisomer (2*S*,4*S*)-4-hydroxyproline (hyp)[13] is known to produce strands that fail to form triple helices. This result could, however, be due to unfavorable steric interactions that develop upon replacing a hydrogen with a hydroxyl group. This suspicion is consistent with molecular modeling of hyp in the Xaa position.[9] Replacing hydrogen with fluorine, on the other hand, typically results in little steric destabilization.[14]

To search for a stereoelectronic effect in the Xaa position on collagen stability, we again used fluorine as a probe, synthesizing the peptides $(FlpProGly)_7$ and $(flpProGly)_7$. Circular dichroism (CD)spectroscopy indicates that $(flpProGly)_7$ but not $(FlpProGly)_7$ forms a stable triple helix at 5 °C (Figure 1A). Moreover, only $(flpProGly)_7$ shows the cooperative transition characteristic of triple-

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Table 1. Effects of 4-Hydroxyproline and 4-Fluoroproline Diastereomers on the Conformational Stability of a Collagen Triple Helix with $(XaaYaaGly)_7$ Strands

	T_m (°C)[a]	
Xaa/Yaa	$(XaaProGly)_7$	$(ProYaaGly)_7$
Flp	no helix	45[b]
Hyp	no helix[c]	36[b]
Pro	6−7[d]	6−7[d]
hyp	no helix[e]	no helix[e]
flp	33	no helix[b]

[a] Temperature at the midpoint of the thermal transition as measured by CD spectroscopy. "No helix" refers to $T_m < 5$ °C. [b] From ref 2. [c] Reported for $(HypProGly)_{10}$ in ref 12. [d] From ref 15. [e] Reported for $(hypProGly)_{10}$ and $(ProhypGly)_{10}$ in ref 13.

helix stability unfolding upon thermal denaturation (Figure 1B). The midpoint of this transition is at 33 °C (Table 1). The linear decrease in elipticity exhibited 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 single strand at 4 °C, whereas both peptides are single strands at 37 °C.

Apparently, stereoelectronic effects can operate adventitiously (or deleteriously) in the Xaa position of collagen (Table 1). 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 hyp in this position.[9] Moreover, the 4*S* substituent in the Xaa position has limited access to solvent, thus making fluorine 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 (Table 1).

The conformational stability of a $(flpProGly)_7$ triple helix is less than that of a $(ProFlpGly)_7$ triple helix (Table 1). Two factors could contribute to this lower stability. First, Flp in the Yaa position causes favorable preorganization of all three main-chain dihedral angles (ϕ, ψ, and ω). In the Xaa position, flp increases the probability of ω adopting a cis ($\omega = 0°$) conformation,[2] thus mitigating somewhat the benefit accrued from the preorganization of ϕ and ψ. Second, a C^γ-endo pucker is already favored in Pro,[7] and flp only increases that preference. In contrast, Flp has the more dramatic effect of reversing the preferred ring pucker, thereby alleviating the entropic penalty of triple-helix formation to a greater degree.

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. 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 crystalline collagen mimic indicates that proline-rich and proline-poor regions have a distinct triple-helical twist,[16] 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.[17]

The development of hyperstable collagens could lead to the creation of new biomaterials for use in medical applications such as wound healing, tissue welding, and tissue engineering.[18] Triple helices formed from proline-rich peptides are more stable than those of proline-poor peptides of comparable size.[19] Thus, the development of hyperstable collagen materials will rely on proline-rich sequences. Herein, we have shown that the conformational stability of these sequences is enhanced by the stereoelectronics of flp in the Xaa position. We anticipate that the rational use of stereoelectronic effects could enhance the stability of other proteins as well.

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Supporting Information Available: Procedures and additional data for syntheses and analyses reported herein (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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