

Effect of fluoro-substituted proline residues on the conformational stability of triple-helical collagen mimics

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Introduction

Collagen is the most abundant protein in animals. In connective tissue, collagen is present as chains wound in tight triple helices, which are organized into fibrils of great tensile strength and thermal stability [1,2]. We have identified a new component of this stability. Collagen consists of XaaYaaGly repeats where Xaa is often L-proline (Pro) and Yaa is often 4(*R*)-hydroxy-L-proline (Hyp). The Hyp residues in a (ProHypGly)_{*n*} triple helix confer substantial additional thermal stability relative to a (ProProGly)_{*n*} triple helix.

It has been proposed that the extra stability ensues from a network of water bridges in which the 4(*R*)-hydroxyl group participates [1,3]. Water bridges that link a Hyp side-chain of one strand to a main-chain carbonyl of another strand have been seen in a crystalline collagen mimic. We believe that water bridges are unlikely to contribute significantly to triple-helical stability [4,5].

We propose instead that the conformational stability of triple helical collagen relies on the electronegativity and stereochemistry of the 4-substituent. To test this hypothesis, we have incorporated 4(*R*)-fluoro-L-proline (Flp) and 4(*S*)-fluoro-L-proline (flp) residues (Fig. 1) into triple helices, which then have (ProFlpGly)₇ and (ProflpGly)₇ strands.

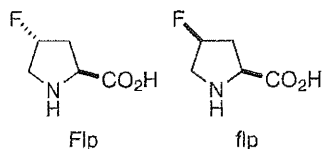


Fig. 1. Structures of 4(*R*)-fluoro-L-proline (Flp) and 4(*S*)-fluoro-L-proline (flp).

Results and Discussion

The conformational stability of a collagen triple helix is indicated by its value of T_m , which is the temperature at the midpoint of the thermal denaturation curve. Values of T_m were acquired by monitoring the change in ellipticity at $\lambda = 225$ nm by circular dichroism spectroscopy or the change in absorbance at $\lambda = 234$ nm by ultraviolet spectroscopy.

We find that *both* the electronegativity and the stereochemistry of the 4-substituent in the Yaa residue is critical for triple helix stability (Table 1). An electronegative 4(*R*)-substituent on a prolyl Yaa residue increases dramatically the T_m of (ProYaaGly)_{*n*} triple helices. Because organic fluorine does not form hydrogen bonds [7], it is unlikely that water bridges are responsible for the increase in thermal stability of (ProFlpGly)_{*n*} triple helices. In contrast, the same substituents in the 4(*S*) configuration are destabilizing. This result leads us to believe that stereoelectronic effects (such as the gauche effect) on the conformation of the pyrrolidine ring have a substantial impact on triple helix stability. We are currently studying the effect of 4-substituents on the *cis*–*trans* equilibrium constant of the prolyl peptide bond and on intramolecular dipole–dipole interactions to reveal the basis for the differences observed in the T_m values.

Table 1. Values of T_m for (ProYaaGly)_n collagen mimics.

Yaa	n	T_m (°C) ^a
4(R)-fluoro-L-proline	7	45
4(R)-hydroxy-L-proline	7	36
4(S)-fluoro-L-proline	7	<10
4(R)-fluoro-L-proline	10	91[4,5]
4(R)-hydroxy-L-proline	10	69[4,5]
L-proline	10	41[4,5]
4(S)-hydroxy-L-proline	10	<5 [6]

^aValues of T_m were determined in 50 mM acetic acid.

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