

Modulating Collagen Triple-Helix Stability with 4-Chloro, 4-Fluoro, and 4-Methylprolines

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Introduction

Collagen is a fibrous protein comprising a right-handed, triple-helical bundle of three parallel, left-handed polyproline II-type helices. Each strand consists of approximately 300 repeats of the trimer (Xaa–Yaa–Gly), where Xaa is often (2*S*)-proline (Pro) and Yaa is often (2*S*,4*R*)-4-hydroxyproline (Hyp) [1]. The most abundant protein in vertebrates, collagen is of fundamental importance to the three-dimensional architecture of such animals. Understanding the chemical determinants of the structure and stability of collagen is essential for both curing collagen-related diseases and creating collagen-based biomaterials.

We have previously demonstrated that pyrrolidine ring pucker and thus triple-helical stability can be manipulated by functionalizing the γ -position of the proline ring with moieties that control the ring pucker via either steric or stereoelectronic effects (Figure 1). The presence of Hyp in the Yaa position of natural collagen greatly increases the stability of the triple helix [2]. This enhanced stability is presumably due to a stereoelectronic effect that defines the pyrrolidine ring pucker of Hyp residues as the C ^{γ} -exo pucker. The C ^{γ} -exo ring pucker fixes the ϕ and ψ angles of Hyp residues to those required for triple-helical assembly [3]. Substitution of non-natural proline derivatives that prefer the C ^{γ} -exo ring pucker, such as (2*S*,4*R*)-4-fluoroproline (Flp) [4] or (2*S*,4*S*)-4-methylproline (Mep) [5], for proline in the Yaa position also stabilizes triple helices. In contrast, the C ^{γ} -endo ring pucker is favorable for triple-helix stability in the Xaa position. Consequently, substitution of (2*S*,4*S*)-4-fluoroproline (flp) and (2*S*,4*R*)-4-methylproline (mep) for proline in the Xaa position stabilizes triple helices, because flp and mep prefer the C ^{γ} -endo ring pucker [5,6].

Here, we introduce additional proline derivatives that can be utilized to endow conformational stability and unique properties on collagen triple helices, namely the 4-chloroprolines. Additionally, we summarize the ability to modulate the thermal stability of collagen over a wide range of temperatures by modifying the γ -carbon of proline residues.

Results and Discussion

We hypothesized that (2*S*,4*R*)-4-chloroproline (Clp) would prefer the C ^{γ} -exo pyrrolidine ring pucker and that (2*S*,4*S*)-4-chloroproline (clp) would prefer the C ^{γ} -endo ring pucker (Figure 1). Thus, Clp should

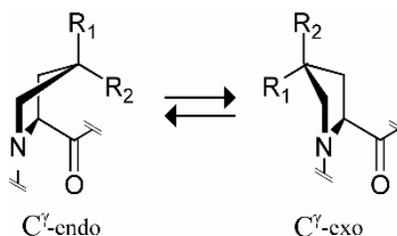


Figure 1. Ring conformations of 4-substituted prolines. The C ^{γ} -endo conformation is favored strongly by stereoelectronic effects when R₁ = H, R₂ = F (flp) or Cl (clp) and by steric effects when R₁ = Me (mep), R₂ = H. The C ^{γ} -exo conformation is favored strongly by stereoelectronic effects when R₁ = OH (Hyp), F (Flp) or Cl (Clp), R₂ = H and by steric effects when R₁ = H, R₂ = Me (Mep).

Table 1. Effect of *clp* and *Clp* on collagen triple-helix stability.

Peptide	T_m
(Pro-Clp-Gly) ₁₀	52
(Pro-Pro-Gly) ₁₀	41
(clp-Pro-Gly) ₁₀	33
(Pro-Clp-Gly) ₇	23
(clp-Pro-Gly) ₇	<10
(Pro-Pro-Gly) ₇	<10
(clp-Clp-Gly) ₁₀	no helix
(Clp-Pro-Gly) ₁₀	no helix

stabilize triple helices in the Yaa position, while *clp* should stabilize triple helices in the Xaa position. To confirm this hypothesis, collagenous peptides containing *clp* and *Clp*

synthesized by a route similar to that of Berger and co-workers [7] were prepared by segment condensation on the solid phase. We tested the triple-helical propensity of *clp* and *Clp* and found that they enable triple helix formation when placed in the Xaa or Yaa position, respectively (Table 1), confirming our hypothesis. (Clp-Pro-Gly)₁₀ and (clp-Clp-Gly)₁₀ do not form triple helices, as expected from prior results for *flp* and *Flp* in collagen mimics [6,8].

Figure 2 illustrates our ability to fine-tune the thermal stability of triple helices of the same length using the results detailed herein and in previous results from our laboratory [3,5,6,8]. Utilizing 4-chloroproline, 4-methylproline, 4-fluoroproline, and combinations of these residues, we have synthesized 21-residue, blunt-ended collagen triple helices with T_m values ranging from 0–53°C. This ability to modulate triple-helical stability by simply modifying the γ -position of proline rings portends the development of synthetic collagen mimetics for applications requiring triple-helical stability over a wide range of temperatures.

Acknowledgments

This work was supported by grant AR44276 (NIH). M.D.S. was supported by a graduate fellowship from the Department of Homeland Security.

References

- Jenkins, C. L., Raines, R. T. *Nat. Prod. Rep.* **19**, 49-59 (2002).
- Sakakibara, S., *et al. Biochim. et Biophys. Acta* **303**, 198-202 (1973).
- DeRider, M. L., *et al. J. Am. Chem. Soc.* **123**, 777-778 (2001).
- Holmgren, S. K., *et al. Nature* **392**, 666-667 (1998).
- Shoulders, M. D., Hodges, J. A., Raines, R. T. *J. Am. Chem. Soc.* **128**, 8112-8113 (2006).
- Hodges, J. A., Raines, R. T. *J. Am. Chem. Soc.* **125**, 9262-9263 (2003).
- Berger, Y., *et al. J. Med. Chem.* **48**, 483-498 (2005).
- Hodges, J. A., Raines, R. T. *J. Am. Chem. Soc.* **127**, 15923-15932 (2005).

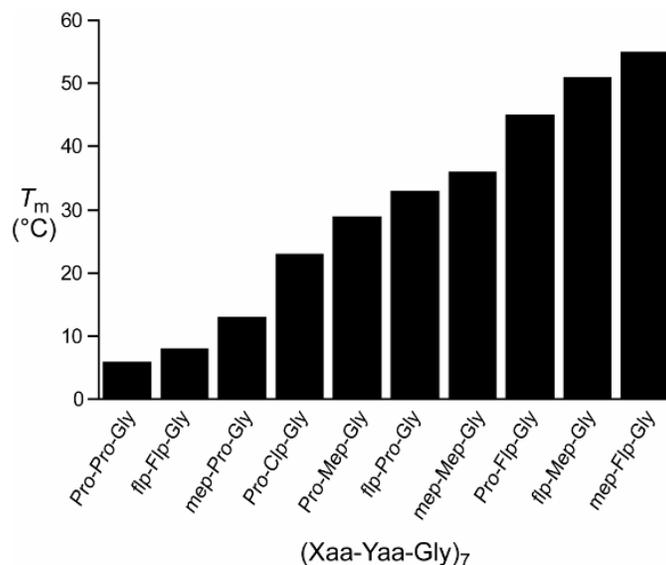


Figure 2. Triple-helix stability can be modulated over a wide range of temperatures by controlling the identity and configuration of functional groups at the γ -position of proline residues [3,5,6,8].