Modulating the conformational stability of triple-helical collagen by chemical modification

Cara L. Jenkins,[1] Kimberly M. Taylor,[2] and Ronald T. Raines[1,3]
[1]Department of Chemistry, [2]Graduate Program in Biophysics, and [3]Department of Biochemistry, University of Wisconsin–Madison, Madison, WI 53706, U.S.A.

Introduction

Collagen is composed of a triple helix of peptides with the sequence $(XaaYaaGly)_n$, where Xaa is often L-proline (Pro) and Yaa is often 4(*R*)-hydroxy-L-proline (Hyp). Each strand of collagen adopts a polyproline-II-like conformation. Natural collagen is found in approximately 19 different types, and is the most prevalent protein in animals. Triple helices comprised of the peptide $(ProHypGly)_{10}$ have been studied extensively as a model for collagen.

Previous work in our laboratory has shown that replacing the Hyp residues in $(ProHypGly)_{10}$ with 4(*R*)-fluoro-L-proline (Flp) residues increases dramatically the value of T_m. For example, in 50 mM acetic acid a $(ProHypGly)_{10}$ triple helix has a T_m (which is the temperature at the midpoint of the thermal transition) of 69 °C, whereas a $(ProFlpGly)_{10}$ triple helix has a T_m of 91 °C [1]. We hypothesize that the greater electron-withdrawing ability of fluorine contributes to the greater conformational stability of $(ProFlpGly)_{10}$.

Results and Discussion

We have modified the hydroxyl groups in $(ProHypGly)_{10}$ with acetyl groups to explore further the contribution of electron-withdrawing ability to conformational stability. The synthesis of $[ProHyp(OAc)Gly]_{10}$ (Fig. 1) was performed using a slightly modified version of the method of Wilchek and Patchornik for selective *O*-acetylation of amino acids [2].

There is one previous report of the preparation of $[ProHyp(OAc)Gly]_{10}$. von Weber and Nitschmann used acetic anhydride as the acetylating agent and trifluoroacetic acid as the solvent. Their measured T_m value for triple-helical $[ProHyp(OAc)Gly]_{10}$ in 1 M NaCl was 25 °C [3]. In our hands, the T_m value for triple-helical $[ProHyp(OAc)Gly]_{10}$ in 50 mM acetic acid is 58 °C, which is approximately 11 degrees lower than that of $(ProHypGly)_{10}$ under the same conditions. Interestingly, the T_m values for triple-helical $[ProHyp(OAc)Gly]_{10}$ and $(ProHypGly)_{10}$ in water are 56°C and 57°C, respectively (Fig. 2). We are in the process of making a series of *O*-acetylated $(ProHypGly)_{10}$ peptides in which the acetyl groups contain one, two, or three fluorines. In this way, we can increase the electron-withdrawing ability of the 4(*R*) substituent inisologous collagen mimics. Moreover, unlike the incorporation of a 4(*R*) fluorine atom [1], these acetylation reactions can be performed on natural collagen with common reagents.

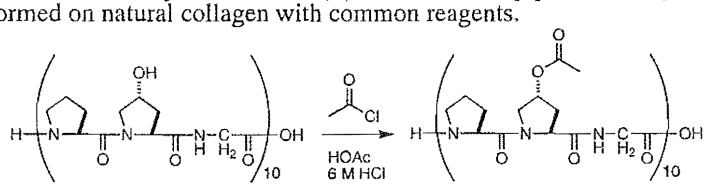

Fig. 1. Synthetic route to $[ProHyp(OAc)Gly]_{10}$.

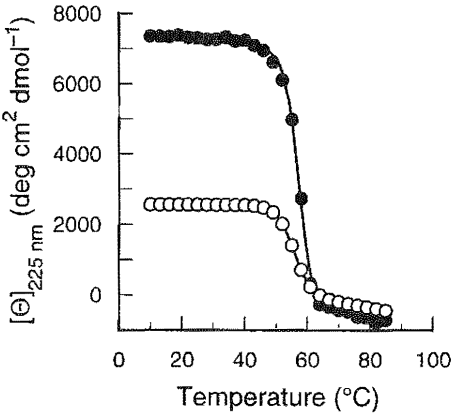

Fig. 2. Effect of temperature on the ellipticity at 225 nm of triple helices of [ProHyp(OAc)Gly]$_{10}$ (closed circles) and (ProHypGly)$_{10}$ (open circles) in water.

Conclusion

The conformational stability of triple helical (ProHypGly)$_{10}$ can be altered by chemical modification of the Hyp hydroxyl group.

Acknowledgments

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