Contribution of mainchain–mainchain hydrogen bonds to the conformational stability of triple-helical collagen

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Introduction

Collagen is the major structural protein in vertebrates. Collagen chains fold into triple-helices of high thermal stability [1,2]. In the current study, we seek to determine the contribution of mainchain–mainchain hydrogen bonds to that stability.

The primary structure of collagen consists of a repeat of the sequence XaaYaaGly, where Xaa is often an L-proline residue and Yaa is often a 4(*R*)-hydroxy-L-proline. The hydroxyl group of Hyp at the Yaa position increases the thermal stability of the triple helix. This thermal stabilization is known to result from an inductive effect of the hydroxyl group [3,4], though it is not yet clear how this inductive effect is conferred.

Within the core of the triple helix lies a ladder of interstrand hydrogen bonds between the N-H of glycine and the C=O of the Xaa residue. The contribution of this prevalent N-H··· O=C hydrogen bond to the thermal stability of the triple helix is unknown. Interestingly, the average N–O interatomic distance between the hydrogen bonding pairs in the crystal structure of $(ProHypGly)_4ProHypAla(ProHypGly)_4$ is 2.87Å [5], which is similar to the length of a hydrogen bond in an α-helix [6], whereas the average N – O distance in $(ProProGly)_{10}$ is 2.96 Å [7], close to the length of an average hydrogen bond [6]. This difference in bond length could reflect a difference in hydrogen bond strength. Here, we test the hypothesis that the hydroxyl group of the Hyp residues increases the thermal stability of the helix by increasing the strength of the N-H··· O=C hydrogen bond.

The relative strength of hydrogen bonds correlates with the ^2H /^1H fractionation factor—the extent to which a particular site becomes enriched in ^2H over ^1H relative to solvent [8]. Stronger hydrogen bonds tend to prefer ^1H over ^2H. In the current study, we observe the equilibrium distribution of ^1H and ^2H in the glycine N-H in AcProGlyOMe and AcHypGlyOMe (which should form hydrogen bonds only with solvent) as well as triple-helical $(ProProGly)_{10}$ and $(ProHypGly)_{10}$.

Results and Discussion

The extent of protonation was measured by NMR spectroscopy. ^1H chemical shifts of the glycine N-H protons were observed at 8.2 ppm for $(ProProGly)_{10}$ and 7.9 ppm for $(ProHypGly)_{10}$. Because most of the 30 glycine residues in each triple helix are in a similar environment, a single N-H peak was observed for each triple helix. It was necessary to carry out the experiments at pH 10.0 due to the extremely slow $^2H/^1H$ exchange of the N-H site in $(ProHypGly)_{10}$ at neutral pH. No significant exchange was observed in $(ProHypGly)_{10}$ after 2 weeks at room temperature, whereas $(ProProGly)_{10}$ achieved equilibrium within 24 h at room temperature.

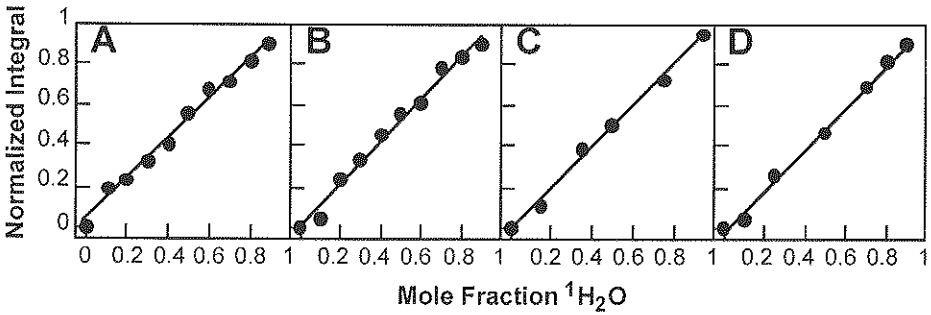

Fig. 1. Results of ^{1}H-NMR experiments monitoring the fraction hydrogen in the glycine NH as a function of ^1H_2O in mixed $^1H_2O/^2H_2O$ solvent. (A) AcProGlyOMe, (B) AcHypGlyOMe, (C) $(ProProGly)_{10}$ triple helix, and (D) $(ProHypGly)_{10}$ triple helix.

Normalized integrals of the glycine N-H peaks as a function of the mole fraction of ^1H_2O versus ^2H_2O are shown in Figure 1. For each peptide (two monomers and two triple helices), the peak volume varies linearly with the ^1H_2O mole fraction. With unusually strong hydrogen bonds, this curve would show an upward bowing [8]. Thus, these results suggest that the interstrand hydrogen bonds in the collagen triple helix are not unusual in strength.

The similarity of the results for AcProGlyOMe, AcHypGlyOMe, $(ProProGly)_{10}$, and $(ProHypGly)_{10}$, suggests that any difference in hydrogen bond strength is too small to detect by measuring deuterium/hydrogen fractionation factors. Moreover, these data do not support the hypothesis that the hydroxyl group of Hyp residues stabilizes the triple helix by increasing the strength of the interstrand hydrogen bond.

Acknowledgments

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