Communication

Characterization of Collagen Model Peptides Containing 4-Fluoroproline; (4(*S*)-Fluoroproline-Pro-Gly) Forms a Triple Helix, but (4(*R*)-Fluoroproline-Pro-Gly) Does Not

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Peptides	Structure	Peptides	Structure
$(Pro\text{-}Pro\text{-}Gly)_{10}$	t		
$(Pro\text{-}Hyp^R\text{-}Gly)_{10}$	t	$(Pro\text{-}fPro^R\text{-}Gly)_{10}$	t
$(Pro\text{-}Hyp^S\text{-}Gly)_{10}$	s	$(Pro\text{-}fPro^S\text{-}Gly)_{10}$	s
$(Hyp^R\text{-}Pro\text{-}Gly)_{10}$	s	$(fPro^R\text{-}Pro\text{-}Gly)_{10}$	s
$(Hyp^S\text{-}Pro\text{-}Gly)_{10}$	s	$(fPro^S\text{-}Pro\text{-}Gly)_{10}$	t

at 4 °C; **t**=triple helix, **s**=single chain.

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Characterization of Collagen Model Peptides Containing 4-Fluoroproline; (4(*S*)-Fluoroproline-Pro-Gly)$_{10}$ Forms a Triple Helix, but (4(*R*)-Fluoroproline-Pro-Gly)$_{10}$ Does Not

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It has been well known that the collagen molecule consists of three polypeptide chains, each of which takes the poly-L-proline II form in a left-handed helix and undergoes a transition to a single coil state as the temperature increases.[1] This characteristic structure is ascribed to the unique amino acid sequence X-Y-Gly, where X and Y are commonly Pro or 4(*R*)-hydroxyproline (4(*R*)-Hyp or Hyp^R). We have shown that (Pro-Pro-Gly)$_{10}$ first synthesized by Sakakibara et al.[2] assumes this collagen structure and shows the thermal transition. Carrying out a series of substitution of Pro with Hyp^R or Hyp^S (4(*S*)-Hyp), we demonstrated that (Pro-Pro-Gly)$_{10}$ and (Pro-Hyp^R-Gly)$_{10}$ have a triple helical structure at room temperature,[3] but (Pro-Hyp^S-Gly)$_{10}$, (Hyp^R-Pro-Gly)$_{10}$, and (Hyp^S-Pro-Gly)$_{10}$ exist in a single stranded state, respectively.[4] These results showed that the stability of the triple helical structure depends on both the stereochemistry and the sequence position of Hyp.

Various studies have been done to explain these results of the substitution. X-ray crystallographic analyses on the single crystals (Pro-Pro-Gly)$_{10}$ and (Pro-Hyp^R-Gly)$_4$-Pro-Hyp^R-Ala-(Pro-Hyp^R-Gly)$_5$ showed that both peptides have a similar main chain conformation, but hydrogen bonds involving hydroxyl groups of Hyp in the latter, which were considered to make a difference in the extent of hydration, cause the difference in the thermal stability.[5] On the other hand, stability from a water bridge was open to question because the transition temperatures of both (Pro-Pro-Gly)$_{10}$ and (Pro-Hyp^R-Gly)$_{10}$ increased extensively in an anhydrous environment.[6] Because a fluorine atom has electron-withdrawing properties and is almost the same size as a hydroxyl group but forms only a weak hydrogen bond when bound to carbon,[7] Raines and co-workers synthesized a polypeptide containing 4(*R*)-fluoroproline (4(*R*)-fPro or fPro^R), (Pro-fPro^R-Gly)$_{10}$.[8] They showed that (Pro-fPro^R-Gly)$_{10}$ displays collagen-like properties. The transition temperature is about 90 °C, which is the highest among known polytripeptides with a similar degree of polymerization.[8] This result indicates that hydrogen bonding does not play an important role. Furthermore, they also synthesized (Pro-fPro^S-Gly)$_7$ (fPro^S: 4(*S*)-fPro) and showed that the transition temperature, if any, is lower than 2 °C.[9] We were interested in the fact that (Pro-fPro^R-Gly)$_{10}$ takes a triple helical structure and (Pro-fPro^S-Gly)$_7$ exists in a random coil state; that is, 4(*R*)-substitution at the Y-position enhances the stability, but 4(*S*)-substitution destabilizes it, and that

this relation is equivalent to the cases of (Pro-Hyp^R-Gly)$_{10}$ and (Pro-Hyp^S-Gly)$_{10}$, respectively. In this work, we synthesized and characterized (fPro^R-Pro-Gly)$_{10}$ and (fPro^S-Pro-Gly)$_{10}$, expecting to obtain further information clarifying the mechanism of stabilization of the triple helical structure.

Syntheses were carried out using Fmoc-protected 4(*R*)- and 4(*S*)-fPro derivatives.[10] The results of sedimentation equilibrium measurements on (fPro^R-Pro-Gly)$_{10}$ and (fPro^S-Pro-Gly)$_{10}$ are shown in Figure 1A. The weight average molecular weights at infinite dilution for (fPro^R-Pro-Gly)$_{10}$ and (fPro^S-Pro-Gly)$_{10}$ were $(2.86 \pm 0.14) \times 10^3$ and $(7.43 \pm 0.37) \times 10^3$, respectively. Because the molecular weight estimated from the chemical composition is 2710.8 for both of them, these values clearly show that (fPro^R-Pro-Gly)$_{10}$ is a monomer and (fPro^S-Pro-Gly)$_{10}$ is a trimer. The small deviations from the linear relation, with the small slope obtained by least-squares fitting, indicate that both peptides exist as monodispersed in solutions. This is confirmed as well by the concentration dependence on the distance from the axis of rotor in each run with residual values (see Supporting Information Figure S1). Circular dichroism (CD) spectra of these two peptides shown in Figure 1B look similar. Yet their temperature dependencies of CD values at around their positive peaks in Figure 1C are significantly different. They indicate that the ellipticity of (fPro^R-Pro-Gly)$_{10}$ decreases linearly as reported for (Hyp^R-Pro-Gly)$_{10}$,[4b] but that of (fPro^S-Pro-Gly)$_{10}$ showed a sigmoidal transition as for (Pro-Hyp^R-Gly)$_{10}$.[3]

We conclude that (fPro^R-Pro-Gly)$_{10}$ exists in a single random coil in the whole range of experimental temperature, but (fPro^S-Pro-Gly)$_{10}$ exists in a triple helical structure at lower temperature and undergoes a thermal transition to a single random coil. Formation of a triple helical structure for (fPro^S-Pro-Gly)$_{10}$ was contrary to our expectation, because (Hyp^S-Pro-Gly)$_{10}$ exists in the single stranded state. The transition temperature, where the fraction of trimer equals 0.5, $T_{1/2}$, for (fPro^S-Pro-Gly)$_{10}$ was 58 °C as shown in Figure 1D. The structures of collagen model peptides are summarized in Table 1.

Many authors including Raines and Zagari have tried to explain the effect of the substitution of Pro by Hyp or fPro on the stability of the triple helical structure.[9,11] Using the results of X-ray analyses on various proline derivatives and collagen model peptides, it was pointed out that the gauche effect (a stereoelectronic effect which is induced by an electronegative element) fixes the pyrrolidine ring puckering,[8a,11] such that Hyp^R and fPro^R prefer the up form,[12a,b] Hyp^S and fPro^S prefer the down form,[10,12b,c] and Pro takes both forms.[11,13] As a general rule of the conformation of the pyrrolidine ring desirable for stabilizing the triple helix, Zagari and co-workers

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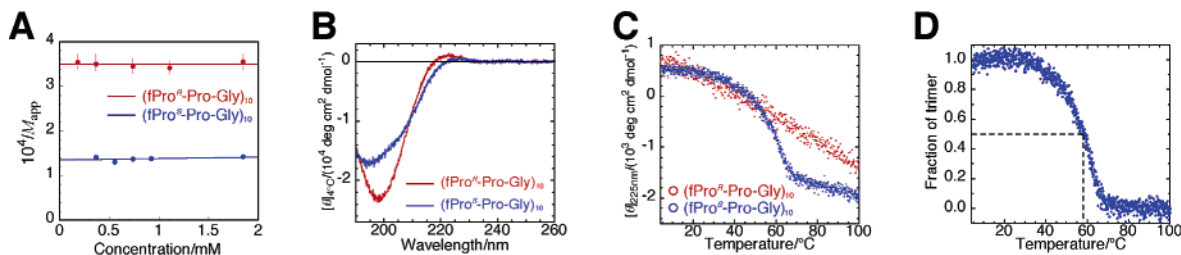
Figure 1. (A) Concentration dependencies of the apparent molecular weights of (fPro^R-Pro-Gly)_{10} and (fPro^S-Pro-Gly)_{10} at 4 °C. (B) CD spectra of (fPro^R-Pro-Gly)_{10} and (fPro^S-Pro-Gly)_{10} at 4 °C. Peptide concentrations were 0.045 mM in 100 mM acetic acid. (C) Temperature dependence of molar ellipticity at 225 nm for (fPro^R-Pro-Gly)_{10} and (fPro^S-Pro-Gly)_{10}. (D) Thermal transition curve for (fPro^S-Pro-Gly)_{10} after the subtraction of baselines for pre- and post-transition states.

Table 1. Summary of the Structures of Collagen Model Peptides

peptides	structure[a]	$T_{1/2}$/°C	refs	peptides	structure[a]	$T_{1/2}$/°C	refs
(Pro-Pro-Gly)_{10}	t	34	3a and 3c				
(Pro-Hyp^R-Gly)_{10}	t	61	3b and 3c	(Pro-fPro^R-Gly)_{10}	t	91[b]	8b and 8c
(Pro-Hyp^S-Gly)_{10}	s	<4	4a	(Pro-fPro^S-Gly)_{10}[c]	s	<2	9
(Hyp^R-Pro-Gly)_{10}	s	<4	4b	(fPro^R-Pro-Gly)_{10}	s	<4	this work
(Hyp^S-Pro-Gly)_{10}	s	<4	4a	(fPro^S-Pro-Gly)_{10}	t	58	this work

[a] At 4 °C; **t** = triple helix, **s** = single chain. [b] From refs 8b and 8c. The temperature was increased by increments of 3 °C with 5-min equilibration. [c] (Pro-fPro^S-Gly)_7 was reported in ref 9. We synthesized and characterized (Pro-fPro^S-Gly)_{10}.

Table 2. Thermodynamic Parameters of the Transition of Collagen Model Peptides

peptides	$T_{1/2}$ /°C	ΔH_{vH} /kJ mol^{-1}	$-T_{1/2}{}^{\star}\Delta S$[a] /kJ mol^{-1}	$\Delta\Delta H_{vH}$[b] /kJ mol^{-1}	$-T_{1/2}{}^{\star}\Delta\Delta S$[b] /kJ mol^{-1}
(Pro-Pro-Gly)_{10}	34	174	−174	0	0
(Pro-Hyp^R-Gly)_{10}	61[c]	220[c]	−188	46	−14
(Pro-fPro^R-Gly)_{10}	80	160	−140	−14	34
(fPro^S-Pro-Gly)_{10}	58	112	−104	−62	70

[a] $T_{1/2}*$ is the transition temperature of (Pro-Pro-Gly)_{10}. [b] $\Delta\Delta H_{vH}$ and $\Delta\Delta S$ are estimated by subtracting these values for (Pro-Pro-Gly)_{10} from those for each peptide, respectively. [c] From ref 14.

thus proposed that the puckering should be down at the X-position and up at the Y-position.[11] However, when (Hyp^S-Pro-Gly)_{10} did not follow this rule, they regarded this as an exception due to the steric interference between the hydroxyl group of Hyp^S and the pyrrolidine ring of Pro in an adjacent chain.[11] Furthermore, our present result that (fPro^S-Pro-Gly)_{10} forms a stable triple helix contradicts these explanations. The triple helix formation of collagen model peptides containing fPro therefore requires an alternative explanation to that applied for the peptides containing Hyp.

To grasp the overall nature of the thermal stability for the peptides, the thermodynamic parameters of the transition, which reflect various factors contributing the triple helix formation, are estimated from the CD transition profile. The van't Hoff enthalpy, ΔH_{vH}, was obtained by the nonlinear least-squares fitting of the CD transition profile. The entropy change of the transition at $T_{1/2}$, ΔS, was calculated by dividing ΔH_{vH} by $T_{1/2}$ (Table 2). The transition temperature of (Pro-Hyp^R-Gly)_{10} is higher than that of (Pro-Pro-Gly)_{10}. This increased stability of (Pro-Hyp^R-Gly)_{10} is ascribed primarily to the $\Delta\Delta H$ because $-T\Delta\Delta S$ is very small. Both of the transition temperatures of (Pro-fPro^R-Gly)_{10} and (fPro^S-Pro-Gly)_{10} are also higher than that of (Pro-Pro-Gly)_{10}. Considering that $\Delta\Delta H$ of these peptides containing fPro is negative, the increased stability is achieved through $-T\Delta\Delta S$. This is a simple explanation that indicates that a hydroxyl group and a fluorine atom affect the stability of a collagen triple helix in different ways. However, it should be noted that errors higher than 10% seem to be unavoidable for parameters derived from the van't Hoff equation. To determine directly the thermodynamic parameters accompanied by the thermal transition, we are now studying with differential scanning calorimetry the collagen model peptides in Table 1.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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