SUPPORTING INFORMATION

Characterization of Collagen Model Peptides Containing 4-Fluoroproline; (4(*S*)-fluoroproline-Pro-Gly)$_{10}$ Forms Triple Helix but (4(*R*)-fluoroproline-Pro-Gly)$_{10}$ Doesn't

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Experimental Procedures

A. Peptides Synthesis and Purification

Model peptides were synthesized using an Applied Biosystems 433A peptide synthesizer. Couplings were carried out on an Alko-PEG resin (Watanabe Chemical Industries, Ltd., 0.26 mmol/g, 0.1 mmol) using Fmoc (9-fluorenyl-methoxycarbonyl) amino acids (4.0 eq). Fmoc-L-proline and Fmoc-glycine were purchased from Peptide Institute Inc. Fmoc-fPro^{R}-OH and Fmoc-fPro^{S}-OH were synthesized by our developed method (See ref 10). The absolute configurations of fPro derivatives were determined by chiral HPLC and X-ray crystallography (See ref 10). HATU (*O*-7-azabenzotriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate) (4.0 eq) / DIEA (*N*,*N*'-diisopropylethylamine) (6.0 eq) mediated peptide couplings. Cleavage of the peptide resin proceeded for 1 h using an

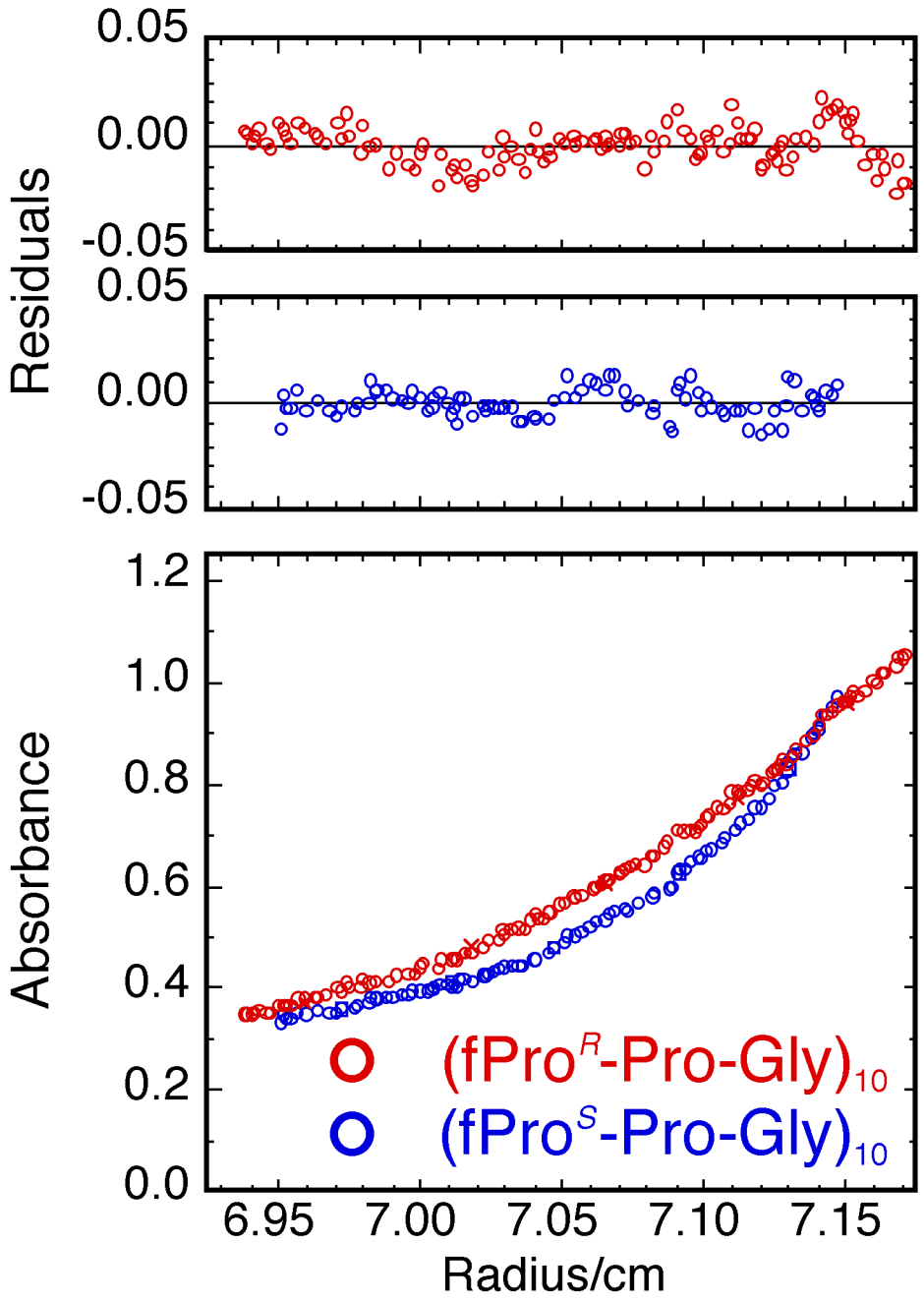
TFA/water/triisopropylsilane mixture (95:2.5:2.5). The peptides were purified by high performance liquid chromatography (HPLC) on a YMC-PACK C-18 reversed-phase column. The peptides were judged by HPLC and MALDI-TOF mass spectrometry to have more than 97% purity.

B. Analytical Ultracentrifugation

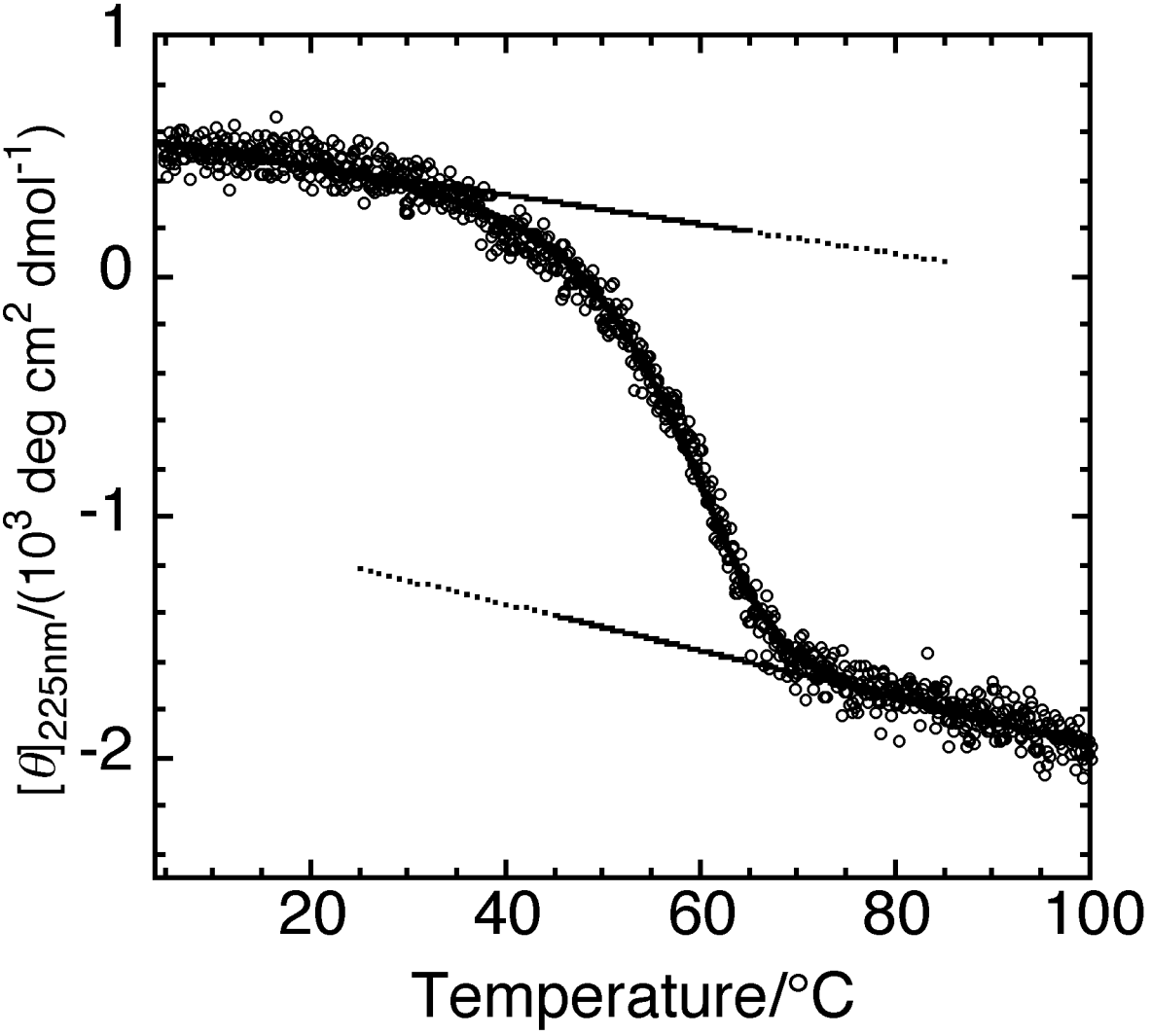
Sedimentation equilibrium studies were performed with a Beckman-Coulter Optima XL-I analytical ultracentrifuge at 4 °C with absorption optics. The rotor speeds for $(fPro^{R}\text{-Pro-Gly})_{10}$ and $(fPro^{S}\text{-Pro-Gly})_{10}$ were 45,000 and 40,000 rpm, respectively. The peptides were dissolved in 100 mM NaCl, 100 mM AcOH at concentrations of 0.18–1.8 mM. Peptide concentrations were determined by measuring the weights of peptides and solutions. Data were collected taking the average of 8 measurements at each radial distance. The partial specific volume of $(fPro^{S}\text{-Pro-Gly})_{10}$ was obtained experimentally to be 0.695 cm^3/g by Anton Paar DMA 5000, and this value is applied for both peptides.

C. Circular Dichroism Spectroscopy

Measurements of circular dichroism spectra were carried out on an Aviv Model 202 spectropolarimeter. Spectra were obtained with a cell of either a 1 or 5 mm path length by averaging 8 scans from 190 to 260 nm. The peptides were dissolved in 100 mM AcOH at concentration of 0.045 mM. Thermal transition curves were obtained by recording the molar ellipticity $[\theta]$ at 225 nm, while the temperature was continuously increased in the range of 4–100 °C at a heating rate of 0.1 °C/min. The fraction of trimer was obtained from the ratio of the difference between the pre-transition baseline and the observed data to the difference between the pre- and post-transition baselines. The result of non-linear least squares fitting employing van't Hoff equation and pre- and post-transition baselines is represented in Figure S2.



Supporting Information Figure S1. Sedimentation equilibrium analyses of $(fPro^{R}\text{-Pro-Gly})_{10}$ and $(fPro^{S}\text{-Pro-Gly})_{10}$ for 1.8 mM solution at 4 °C. The rotor speeds for $(fPro^{R}\text{-Pro-Gly})_{10}$ and $(fPro^{S}\text{-Pro-Gly})_{10}$ were 45,000 and 40,000 rpm, respectively. The sedimentation profiles were obtained at 245 nm for $(fPro^{R}\text{-Pro-Gly})_{10}$ and 243 nm for $(fPro^{S}\text{-Pro-Gly})_{10}$.



Supporting Information Figure S2. Temperature dependence of molar ellipticity at 225 nm for (fPro^S-Pro-Gly)$_{10}$ with pre- and post-transition baselines. The result of non-linear least squares fitting with van't Hoff equation is also shown as a solid line.