Molecular dependence of collagen model peptide studied by low frequency Raman spectroscopy

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Introduction: Low frequency Raman spectra reflects the intermolecular vibration and the interactions between molecular chains. Especially, low frequency Raman spectroscopy is a powerful tool for monitoring higher-order structures of polymer in water. Therefore, we tried to investigate the structural changes of collagen model peptide by low frequency Raman spectroscopy.

Experimental Section: Two kinds of collagen model peptides ((Pro-Pro-Gly)₁₀ (PPG10) and (Pro-Pro-Gly)₅ (PPG5)) are used as samples (Figure 1). PPG10 and PPG5 are dissolved in D₂O. HR-800-LWR (HORIBA) and SureBlock XLF (Ondax) are used for this study. Each range of measurements are from 70 cm⁻¹ to 3200 cm⁻¹ and from 20 cm⁻¹ to 1590 cm⁻¹. T95-HS (LINKAM) is used as a temperature controller.

Results and Discussion: PPG10 forms a triple-helical structure like collagen in aqueous solution, and denatures when it is heated. This structural change from a triple-helix to a random coil in aqueous solution occurs reversibility in PPG10[1]. However, PPG5 does not show such a conformational change[2]. Figure 2 shows temperature dependent low frequency Raman spectra and their second derivative of PPG10/D₂O and PPG5/D₂O from 27.8 °C to 63.2 °C. They were given by denoising by singular value decomposition, curve fitting, normalization, and subtraction (each data – 70 °C). There are three peaks appeared at around 259 cm⁻¹, 178 cm⁻¹, and 106 cm⁻¹ in the 80-300 cm⁻¹ region. Figure 3 shows the change of the peak position at around 178 cm⁻¹ and 106 cm⁻¹. During cooling process, the peak at around 178 cm⁻¹ of PPG10 showed a shift to lower frequency region. While that of PPG5 did not change with temperature (Figure 3 (a)). Moreover, the peak at around 106 cm⁻¹ of PPG10 shifted to the higher frequency region, while that of PPG5 did not change with temperature (Figure 3 (b)). It is already reported that the peak at 178 cm⁻¹ due to the water and 106 cm⁻¹ reflected to the intermolecular hydrogen bond in the amide group[3,4]. Therefore, the
lower frequency shift of the peak at 178 cm\(^{-1}\) due to water indicated that the hydrogen bond between water-to-water is disturbed by interaction between collagen model peptide and water when temperature becomes higher than the coil-helix transition temperature, but it is not interfered when temperature becomes lower than the temperature which is the formation of triple-helical structure. And the higher frequency shift of 106 cm\(^{-1}\) indicated that the intermolecular hydrogen bond in the amide group of the collagen model peptide is stronger because triple-helical structure is formed. These results show that the triple-helical structure of collagen model peptide are formed between 40 to 50 °C. We were able to demonstrate that structural changes from helix to random coil by low frequency Raman spectroscopy.

References:


![Chemical structure of PPG10 (n= 10) and PPG5 (n= 5)](image1)

**Figure 1** Chemical structure of PPG10 (n= 10) and PPG5 (n= 5)

![The Raman spectra and their 2nd derivative spectra of (a) PPG10 (b) PPG5](image2)

**Figure 2** The Raman spectra and their 2nd derivative spectra of (a) PPG10 (b) PPG5

![The peak position of PPG10 and PPG5 at around (a) 178 cm\(^{-1}\) (b) 106 cm\(^{-1}\)](image3)

**Figure 3** The peak position of PPG10 and PPG5 at around (a) 178 cm\(^{-1}\) (b) 106 cm\(^{-1}\)