Crystallization of (Pro-Hyp-Gly)$_{10}$ and Its Triple-Helical Structure Deduced from Cylindrical Patterson Map

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Two morphologically different crystals of a collagen model peptide (Pro-Hyp-Gly)$_{10}$ were obtained using PEG 200 as the precipitating agent. Both the crystals belonged to a monoclinic system with the space group $P2_1$ and diffracted to a resolution of 1.8 Å. Although the crystal data were quite different from those of homologous model peptide, (Pro-Pro-Gly)$_{10}$, the cylindrical Patterson map showed that the main chain conformation of this compound is exactly the same as that of (Pro-Pro-Gly)$_{10}$. This result supports the Okuyama model for collagen structure.

Triple helix is an unique structural motif found in the fibrilar forming collagens and a range of other extracellular matrix protein, a series of host defense proteins and certain membrane proteins.[1] The basic structure of collagen was found to be a triple-helix, due to the strict sequence constraints, with glycine as every third residue and a high content of imino acids. For the detailed conformation of collagen, however, two models have been generally accepted. One is the Rich and Crick model in which each strand has a 10/1-helical symmetry and a helical pitch of 86 Å.[2] This was proposed based on the fiber diffraction pattern from native collagen. The other is the Okuyama model in which each strand has a 7/1-helical symmetry and a 60 Å helical pitch.[3,4] The latter model was obtained by the structure analysis of (Pro-Pro-Gly)$_{10}$ single crystals and this model could also explain the fiber diffraction pattern of native collagen.[5] That is, both the models could be proposed from the diffraction pattern of native collagen with equal plausibility. Recently the crystal structure analysis of (Pro-Hyp-Gly)$_{10}$ in which one of the Gly is changed to Ala has been solved and this structure was in accordance with the Okuyama model except for the region around Ala.[6] To get better understanding of collagen structure, we are studying crystal structures of collagen model peptides. In this paper, one such triple-helix forming peptide, (Pro-Hyp-Gly)$_{10}$, has been discussed.

(Pro-Hyp-Gly)$_{10}$ peptide was obtained from Peptide Institute Inc., Osaka, Japan and was used for crystallization experiments without any further purification. The crystals suitable for crystallographic analyses were grown by the hanging drop method at 10 °C. The drop consists of the peptide solution at a concentration of 4 to 5 mg/ml, 10% acetic acid and 11% PEG 200. About 1 ml of PEG200 at a concentration of 22% was used as the reservoir solute. As shown in Figure 1, crystals were obtained with two different morphologies, namely, triangular and rectangular in the same drop. The triangular crystals grew to a size of 0.3 x 0.3 x 0.2 mm^3, while the rectangular crystals grew to a size of 0.4 x 0.3 x 0.2 mm^3. These crystals were used for the preliminary analyses and data collection.

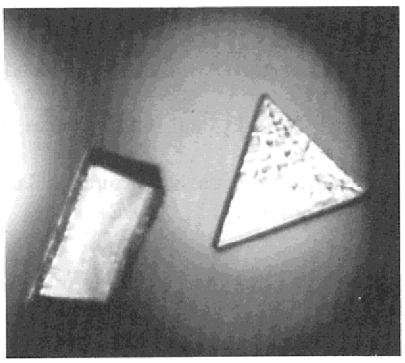

Figure1. Two types of (Pro-Hyp-Gly)$_{10}$ single crystals found in the same drop.

The precession photograph along the 0kl plane is shown in

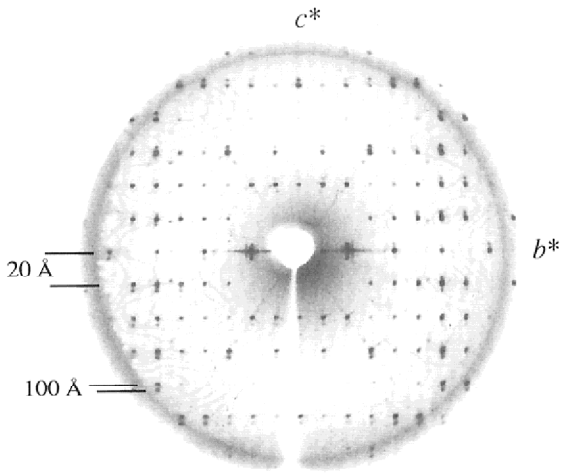

Figure 2. 0kl precession photograph of (Pro-Hyp-Gly)$_{10}$.

Figure 2, which was taken using a Rigaku ultraX18 rotating anode generator (40 kV, 150 mA) with CuKα radiation, with μ=15°. In this photograph, very bright spots corresponding to 20 Å apart (l = 5n) can be clearly seen. In addition to these, one or two spots (satellite spots) can also be seen on either side of these bright spots. The separation between these are found to be 100 Å. This kind of satellite spots was also observed in the precession photograph of (Pro-Pro-Gly)$_{10}$.[7] Rigaku, four-circle diffractometer (AFC5R) with CuKα radiation, generated by a Rigaku RU200 rotating anode (40 kV, 100 mA) was used for the evaluation of the cell parameters and intensity data collection. Both the crystal types belonged to a monoclinic system with the space group $P2_1$ and the cell parameters being a=14.04(1), b=26.60(2), c=100.30(3) Å and β=106.89(5)°. Both the morphologically different crystals were used for data collection which was performed at 5 °C, due to the rapid decay of the crystal at room temperature. In all, four crystals were used to collect data up to a resolution of 1.8 Å.

The two-dimensional Patterson map was calculated from hk0

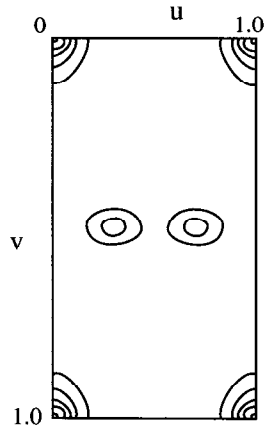

Figure 3. Two-dimensional Patterson map of $(Pro\text{-}Hyp\text{-}Gly)_{10}$.

reflections (Figure 3). The vector peak at (u =0.31, v =0.5) shows that in the *ab*-plane there are two molecular rods perpendicular to this plane and separated by 15 Å. This was confirmed by the cylindrical Patterson map (Figure 4) calculated from 1166 reflections with l=5n (n=0, 1, 2, ···). The other reflections (l≠5n) were not considered since many of them were too weak. In this map, vector peaks within the molecular rod were observed along the Z-axis within the region of r<8 Å, while those between molecular rods were observed as continuously aligned vertical peaks around r=15 Å. Details of vector peaks within a molecular rod are exactly same as those observed in that of $(Pro\text{-}Pro\text{-}Gly)_{10}$,[7] which suggested the conformational similarities of these two compounds. Therefore, from these Patterson maps, following results were deduced. The unit cell contains two $[(Pro\text{-}Hyp\text{-}Gly)_{10}]_3$, in which each strand has a 7/1 helical symmetry within a helical pitch of 60Å and three such strands make a triple-helical structure. Because of the geometrical relation among three strands, the repeating period of the triple helix is reduced to one third of the pitch length as shown in the schematic illustration of the triple-helical model of $(Pro\text{-}Hyp\text{-}Gly)_{10}$ (Figure 5). These results confirm that the overall conformation of $(Pro\text{-}Hyp\text{-}Gly)_{10}$ is exactly same as that of the Okuyama model for collagen. Further studies are in progress.

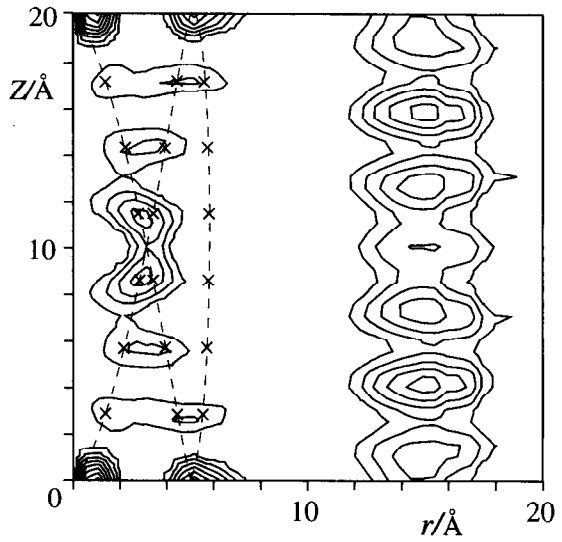

Figure 4. Cylindrical Patterson map of $(Pro\text{-}Hyp\text{-}Gly)_{10}$. The crosses denote the positions of the expected vector peak between helical units when each strand in the triple helix has a 7/1-helical symmetry and a helical pitch of 60 Å.

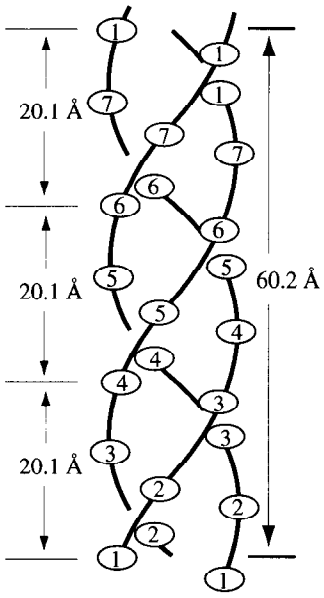

Figure 5. Schematic illustration of the triple-helical structure of $(Pro\text{-}Hyp\text{-}Gly)_{10}$. Each ellipse represents Pro-Hyp-Gly unit. The number in the ellipse denotes the unit number of the corresponding peptide chain.

References

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