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Average Crystal Structure of (Pro-Pro-Gly)$_9$ at 1.0 Å Resolution

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ABSTRACT: The average crystal structure of a collagen-model peptide, (Pro-Pro-Gly)$_9$ has been determined at 1.0 Å resolution. Crystals belong to an orthorhombic system ($P2_12_12_1$) with cell parameters of a = 26.82(2), b = 26.33(2), and c = 20.25(2) Å. The X-Ray oscillation photograph clearly showed satellite spots with an 80 Å axial repeat on both sides of the strong spots on the layer lines corresponding to c = 20 Å. The existence of c = 80 Å axial repeat suggests that the longer repeat along the c-axis is the shortest integral multiply of the helical repeat (20 Å) enough to accommodate one (Pro-Pro-Gly)$_9$ molecule and an appropriate gap between adjacent molecules. According to the reflection data with c = 20 Å axial repeat, the overall peptide structure is very close to the left-handed 7/2-helical model for collagen. Based on the difference Fourier map, 34 water molecules were added in the asymmetric unit of seven triplets. Of which 14 water molecules make hydrogen bonds with peptide chains and rest of them participate in hydrogen bonds only with other water molecules.

KEY WORDS Collagen / Triple Helix / Crystal Structure / Pro-Pro-Gly / Single Crystal /

Collagen molecule is the major fibrous element of skin, bone, tendon, cartilage, teeth, and so on. It is found in at least small quantities in most animal phyla and is very important in the vertebrates where roughly a third of the total body protein is its major structural constituent. Because of the strict sequence constraints with glycine (Gly) as every third residue, the amino acid sequence of collagen is usually assumed to be (Gly-Xaa-Yaa)$_n$. Here, Xaa and Yaa positions are frequently occupied by the imino acids, proline (Pro) and 4-hydroxyproline (Hyp) respectively. Due to the above characteristic features in the amino acid sequence, collagen molecule has a triple-helical structure, in which the Gly residue is located near the helical axis and the other amino acids (Xaa and Yaa) are outside. Each of the three strands has a left-handed polyproline II type 3/1-helical conformation. Such three strands wind around a common axis in a right-handed fashion. There are two models with different helical symmetry and axial repeat which have been generally accepted as a collagen structure. One is the Rich and Crick model in which three strands form a left-handed 10/3-helix with an axial repeat 28.6 Å[1] as a whole. Each strand forms a right-handed 10/1-helix with ten Gly-Xaa-Yaa units and a pitch length of 85.8 Å (= 28.6 × 3). This model was proposed based on the fiber diffraction pattern of native collagen. The other is the Okuyama model in which three strands form a left-handed 7/2-helix with an axial repeat 20.0 Å[2] as a whole. Each strand forms a right-handed 7/1-helix with seven triplets and a pitch length of 60.0 Å (= 20.0 × 3). This triple-helical structure was found in the single crystal of (Pro-Pro-Gly)$_{10}$[3,4] (hereafter, PPG10). Since this structure could explain main features in the fiber diffraction pattern of native collagen, it was proposed as a collagen model structure.[2] In the earlier studies, the helical asymmetric unit of PPG10 was determined by using Linked-Atom Least-Squares method[5] for fibrous polymers. Thus-obtained structure is referred to as PPG10-0.[4]

Recently the structure of PPG10 was reanalyzed by the method used for protein single crystals.[6,7] Furthermore, several single crystals of collagen model peptides, such as (Pro-Hyp-Gly)$_{10}$,[8] (Pro-Hyp-Gly)$_4$-Pro-Hyp-Ala-(Pro-Hyp-Gly)$_5$[9] and (Pro-Hyp-Gly)$_3$-Ile-Thr-Gly-Ala-Arg-Gly-Leu-Ala-Gly-Pro-Hyp-Gly-(Pro-Hyp-Gly)$_3$[10] and (Pro-Hyp-Gly)$_4$-Glu-Lys-Gly-(Pro-Hyp-Gly)$_5$[11] have been analyzed. Hereafter, these are referred to as PPG10-1,[6] PPG10-2,[7] POG10,[8] (Gly→Ala) peptide,[9] T3-785 peptide,[10] and EKG peptide,[11] respectively. The (Gly→Ala) peptide has Pro-Hyp-Gly motifs with a single substitution of Gly to Ala at the central triplet. The T3-785 peptide consists of 12 amino acid residues in the sequence from 785 to 796 of human type III collagen and (Pro-Hyp-Gly)$_3$ at both ends in order to hold its triple-helical structure. The sequence included in this peptide occurs one triplet C-terminal to the unique collagenase cleavage site and contains continuous nine residues with no imino acids. Except the region close to the Ala substitution

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site of (Gly→Ala) peptide and the central region of T3-785 peptide, every triple-helical conformation in these crystals was found to be very close to that of the Okuyama model rather than the Rich and Crick model for collagen.

In the precession and oscillation photographs of PPG10[4,6] and POG10,[8] weak satellite diffraction spots were observed on either side of strong diffraction spots corresponding to a 20 Å repeat along the *c*-axis. Since these satellite spots were assumed to be 100 Å axial repeat,[4,6–8] at first, layer lines with 20 Å repeat were designated as $l = 5n$ (n; integer) and those of satellite spots as $l = 5n + m$ ($m = 1, 2, 3, 4$). Recently, however, we found that some of these satellite spots could not be explained by a 100 Å axial repeat when examined the enlarged diffraction pattern recorded on an imaging plate. Furthermore, PPG10 crystals grown under microgravity in the Space Shuttle revealed 182 Å repeat along the *c*-axis instead of 100 Å.[12] Very recently, the structure of thus-obtained PPG10 crystal was analyzed for the $c = 20$ Å cell.[13] Since we thought that the molecular length of model peptide has a close relationship with this long axial repeat, we synthesized (Pro-Pro-Gly)$_9$ and (Pro-Pro-Gly)$_{11}$ (hereafter, PPG9 and PPG11, respectively) in order to clarify the physical meaning of satellite spots. In this study we report the crystal structure of PPG9 at 1.0 Å resolution, together with physical meaning of satellite spots that appear in the diffraction patterns of (Pro-Pro-Gly)$_n$ (n = 9, 10, 11) and (Pro-Hyp-Gly)$_n$ (n = 10, 11).

EXPERIMENTAL

Peptide Synthesis and Crystallization

Peptides were synthesized by solid-phase method on phenylacetamidemethyl (PAM) resin[14] with *tert*-butyloxycarbonyl (Boc) chemistry. Boc-Gly-OH was attached to the resin and chain elongation was performed by fragment condensation using Boc-Gly-Pro-Pro-OH that was first established by Sakakibara *et al*.[15] At each segment condensation, 2.0 eq. of building blocks were used with the 2-(1-hydroxybenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/1-hydroxybenzotriazole (HOBt) as a coupling reagents. Removal of the Boc group was performed by 25% trifluoroacetic acid (TFA) in dichloromethane treatment after each coupling step. The Kaiser ninhydrin test was used to monitor the coupling reaction.[16] Finally, Boc-Pro-Pro-OH was condensed to complete the objective peptide sequence. The peptide resin was treated with anhydrous HF to release the peptide from the resin. The crude product was purified by Sephadex G-50 (40% acetic acid) and corresponding fractions were collected, concentrated and lyophilized. Fast atom bombardment mass (FAB-MS) spectra gave corresponding peak.

The crystals suitable for crystallographic analyses were grown by the hanging drop method at 11°C within a week. The peptide solution consists of PPG9 at concentrations of 4.5 mg mL^{-1}, 5% (v/v) acetic acid and 0.5% (w/v) sodium azide. As a reservoir solute, 1 mL of 20% PEG 400 was used. A mixture made up of 3 μL of peptide solution and 3 μL of reservoir solution was used as a crystallization drop.

Determination of Lattice Constants and Data Collection

The crystal sealed in a glass capillary along with the mother liquor was used for measurement by a 4-circle diffractometer (AFC5R, Rigaku Co.) with graphite monochromatized Cu-K_α radiation from the rotating anode generator (RU 200, Rigaku Co.) operated at 40 kV and 150 mA. Still and oscillation photographs of the crystal were also taken using an imaging plate (Raxis-IV, Rigaku Co.) with graphite monochromatized Cu-K_α radiation from the rotating anode generator (ultraX18, Rigaku Co.) operated at 50 kV and 250 mA. These diffraction photographs indicated the presence of satellite spots on either side of the reflections corresponding to the 20 Å axial repeat, which was similar to those observed in PPG10 and POG10 but with different long axial repeat.

The intensity data corresponding to the $c = 20.25$ Å unit cell was collected at 20°C using the 4-circle diffractometer with ω scans up to a resolution of 0.9 Å. Intensities for 10330 reflections were collected and corrected for the Lorentz-polarization and absorption effects. Data collection parameters and refinement statistics are shown in Table I.

Structure Determination and Refinement

The structure has been solved by the molecular replacement method with the same assumption as those in the previous studies[6–8] that the molecule has an infinite helical conformation. The probe was based on the structural model for collagen proposed by Okuyama,[4] which consists of three strands having 7 amino acid residues each and as a whole consists of 7 triplet units with a left-handed 7/2-helical symmetry within a 20 Å axial repeat. The probe model was placed at the appropriate position in the unit cell using X-PLOR.[17] A total of 2922 unique reflections with ($Fo > \sigma(Fo)$) in the resolution range 8.0 to 1.0 Å was used for the structure determination and refinement, of which 292 reflections were used to monitor the *freeR*. The refinement of the structure was done by SHELX-97.[18] Restraints for

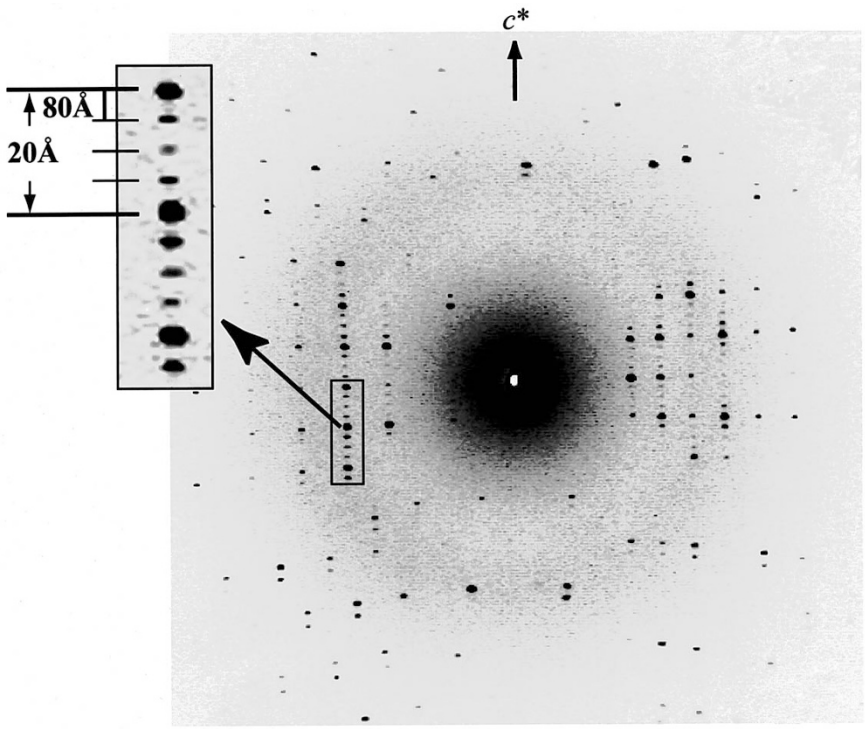

Figure 1. Oscillation photograph of (Pro-Pro-Gly)$_9$ recorded on an imaging plate. Between strong reflections with a 20Å axial repeat, many reflections corresponding to an 80 Å axial repeat can be seen.

Table I. Data collection parameters and refinement statistics of (Pro-Pro-Gly)$_9$

Data collection device	AFC5R-Rigaku 4-Circle diffractometer
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Cell dimensions	
a/Å	26.82(2)
b/Å	26.33(2)
c/Å	20.25(1)
(c'/Å	81.00)
Volume/Å^3	14300(29)
Scan mode	ω
Scan speed/°min^{-1}	8
Scan width/°	$(\Delta\omega) = (1.10 + 0.3 \tan\theta)$
$2\theta_{max}$/°	120
Range of h, k, l	$0 \leqq h \leqq 30,\ 0 \leqq k \leqq 30,$ $0 \leqq l \leqq 23$
Resolution range/Å	8.0–0.9
Data collection temp./°C	20
No. of reflections measured	10651
No. of unique reflections	10330
No. of reflections usec for refinement ($F > \sigma(F)$)	2992
R-factor	22.2%
freeR-factor	29.4%
Introduced water molecules /7 triplets	34

bond lengths and bond angles were applied during the refinement, while no torsion angle restraints were applied. Certain constraints were imposed in order to ensure the continuity of the triple-helix belonging to the adjacent unit cells along the *c*-axis. The structure was refined for several cycles and corrections were carried out based on the 2*Fo-Fc* electron density map using the molecular graphics program Xfit of the software package XtalView.[19] Water molecules were picked based on the *Fo-Fc* electron density map.

RESULTS AND DISCUSSION

Crystal Data

Single crystals of PPG9 were rectangular in shape with dimensions of approximately $0.3 \times 0.3 \times 0.3\text{mm}^3$. As shown in an oscillation photograph (Figure 1), strong diffraction spots corresponding to a 20 Å axial repeat can be clearly observed along the c^*-axis. In addition to these, rather weak satellite spots with an 80 Å axial repeat can be seen at least on one side, and in many cases both sides of the strong spots with a 20 Å axial repeat. Therefore, layer lines with 20 Å repeat can be designated as $l = 4n\,(n = 1, 2, 3, \cdots)$, while those of the satellite spots $l = 4n + m\,(m = 1, 2,$ and $3)$. In some cases three satellite spots between $l = 4n$ and $4(n + 1)$ are observed clearly. However, the total number of observed satellite spots is rather small compared with that of the expected ones. Furthermore, their intensities are very weak compared with those of $l = 4n$

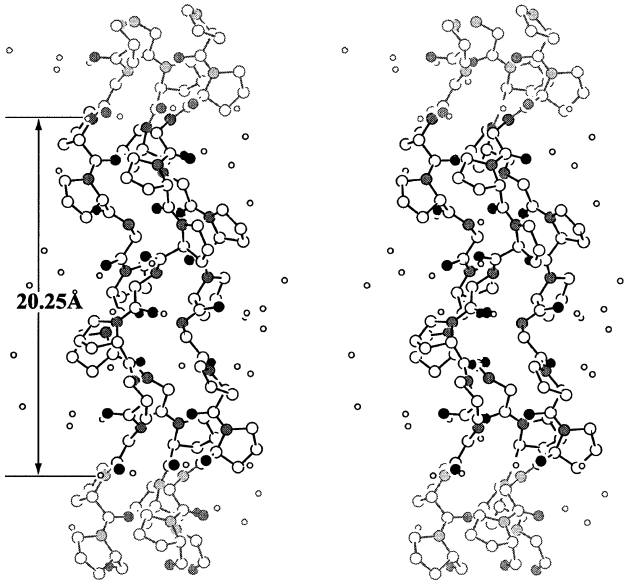

Figure 2. Stereo-view of the (Pro-Pro-Gly)$_9$ triple-helical structure. Small open circle denotes water molecule which is hydrogen bonded to the peptide and/or other water molecules. Deep and light gray circles denotes oxygen and nitrogen atoms, respectively. The figure was generated with ORTEP.[30]

reflections. Therefore, in this study satellite spots were not used in the structure analysis. That is, only the structure of seven triplets in an asymmetric unit was analyzed. This situation is the same as those in the cases of PPG10[6,7] and POG10.[8] The crystal has an orthorhombic system ($P2_12_12_1$) with cell dimensions of a = 26.82(2), b = 26.33(2), and c = 20.25(1) Å.

Molecular Conformation

The final structure contains 21 amino acid residues (126 non-hydrogen atoms) and 34 water molecules (R = 0.222, *freeR* = 0.294) and its peptide conformation is shown in Figure 2. As observed in the Okuyama model and the Rich and Crick model, it was found in the present structure that three strands are held together by hydrogen bonds between the NH of Gly in one strand and the O=C of the Pro(X) in the next strand (Table II). In addition to this, carbonyl oxygens of Pro(X) and Gly are close to the Cα of Pro(Y) and Gly, respectively. These may be regarded as the C–H$\cdots$O hydrogen bond. These features were also found in the structure of PPG-0,[4] PPG10-1,[6] PPG10-2,[7] POG10,[8] T3-785 peptide,[10] EKG peptide[11] and also those of (Gly→Ala) peptide[9] except for the region close to the Ala substitution site. Historically, one of these C–H$\cdots$O hydrogen bonds was first proposed by Ramachandran and coworkers as a necessity for the stability of their triple-helical structure.[20]

The average ϕ, ψ, and ω angles of the Pro(X), Pro(Y) and Gly are (−76.6°, 163.2°, −178.9°), (−60.8°, 153.4°, 176.9°) and (−74.6°, 176.7°, 177.1°), respectively. These are very close to those in the previous studies (Table III). The helical parameters (unit height and unit twist) for each triplet were calculated from the bond lengths, bond angles, and torsion angles (ϕ, ψ, and ω) by the method of Sugeta and Miyazawa.[21,22] The average unit height and unit twist of PPG9 are 8.52 Å and 51.0°, respectively. These values are very close to those of the previous studies and those of the ideal 7/2-helical model (Okuyama model), but very different from those of the Rich and Crick model (Table III). According to our preliminary analysis of PPG11, this peptide also has a conformation very close to the ideal 7/2-helix. Furthermore, the recent structural studies on collagen model peptides tell us that the 7/2-helical conformation is more favourable in the imino acid rich region and Gly-Xaa-Yaa triplet sandwitched by (Gly-Pro-Pro)$_n$ or (Gly-Pro-Hyp)$_n$. From these results we can conclude that the molecular length of peptide does not affect the triple-helical conformation. This conclusion seems to be very reasonable. However, it is inconsistent with

Table II. Inter-strand hydrogen bonding parameters

N–H$\cdots$O hydrogen bonds involving N of Gly and O of Pro(X)

Hydrogen bonds	N$\cdots$O/Å	H$\cdots$O/Å	<N–H$\cdots$O/°
N(203)$\cdots$O(301)	2.97	2.20	149.8
N(303)$\cdots$O(104)	2.93	2.16	149.9
N(106)$\cdots$O(204)	2.96	2.16	156.0
N(206)$\cdots$O(304)	2.91	2.08	161.8
N(306)$\cdots$O(107)	3.03	2.24	151.5
N(109)$\cdots$O(207)	3.00	2.18	160.4

Cα–H$\cdots$O hydrogen bonds involving Cα of Pro(Y) and O of Pro(X)

Hydrogen bonds	C$\alpha\cdots$O/Å	H$\cdots$O/Å	<Cα–H$\cdots$O/°
Cα(202)$\cdots$O(301)	3.17	2.30	146.2
Cα(302)$\cdots$O(104)	3.28	2.44	143.9
Cα(105)$\cdots$O(204)	3.36	2.55	140.4
Cα(205)$\cdots$O(304)	3.30	2.47	141.9
Cα(305)$\cdots$O(107)	3.28	2.42	145.9
Cα(108)$\cdots$O(207)	3.38	2.53	160.4

Cα–H$\cdots$O hydrogen bonds involving Cα of Gly and O of Gly

Hydrogen bonds	C$\alpha\cdots$O/Å	H$_1\cdots$O/Å H$_2\cdots$O/Å	<Cα–H$_1\cdots$O/° <Cα–H$_2\cdots$O/°
Cα(203)$\cdots$O(103)	3.12	2.56	116.6
		2.84	97.1
Cα(303)$\cdots$O(203)	3.06	2.50	116.5
		2.78	97.2
Cα(106)$\cdots$O(303)	3.06	2.56	111.6
		2.71	101.7
Cα(206)$\cdots$O(106)	3.15	2.70	109.4
		2.79	103.1
Cα(306)$\cdots$O(206)	3.12	2.61	113.4
		2.81	99.3
Cα(109)$\cdots$O(306)	3.09	2.54	115.9
		2.79	98.4

Table III. Average (ϕ, ψ, ω) conformation angles and average hlical parameters (h, θ)

	PPG9	PPG10-1	PPG10-2	POG10	(Gly→Ala)	T3-785	Idial helical model	
							7/2-helix	10/3-helix
Torsion angle								
ϕ(X)/°	−76.6	−77.1	−75.0	−72.7	−72.6	−73.2	−75.5	−72.1
ψ(X)/°	163.2	163.8	161.4	161.1	163.8	159.4	152.0	164.3
ω(X)/°	−178.9	179.1	177.8	179.6	179.9	179.6	−176.8	180.0
ϕ(Y)/°	−60.8	−62.4	−61.2	−58.4	−59.6	−62.7	−62.6	−75.0
ψ(Y)/°	153.4	154.0	153.3	152.0	149.8	149.1	147.2	155.8
ω(Y)/°	176.9	177.6	176.7	178.5	178.5	179.9	172.8	180.0
ϕ(Gly)/°	−74.6	−76.7	−75.8	−74.8	−71.9	−69.1	−70.2	−67.6
ψ(Gly)/°	176.7	176.6	179.5	172.8	174.1	170.9	175.4	151.4
ω(Gly)/°	177.1	179.0	−179.9	179.2	177.3	179.7	178.2	180.0
Unit height, h/Å	8.52	8.79	8.74	8.45	8.44	8.5	8.61	8.58
Unit twist, θ/°	51	50.7	52.6	46.5	57.1	46.7	51.4	36

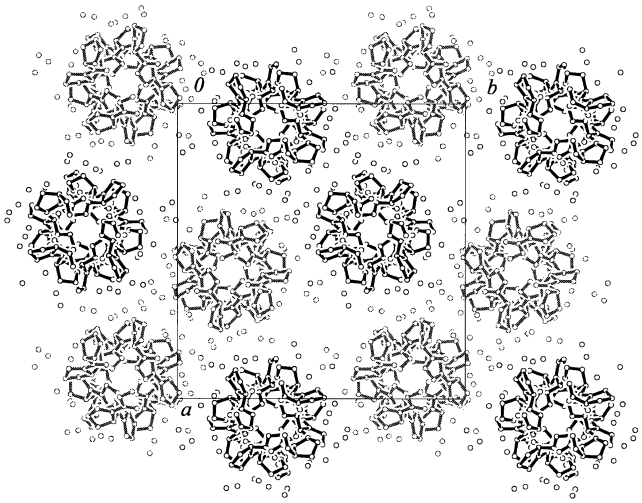

Figure 3. Lateral packing of (Pro-Pro-Gly)$_9$ together with water molecules between triple-helices. Water molecules are represented by small open circles.

the result reported for poly(Pro-Gly-Pro),[23] in which polymer chains were assumed to be a 10/3-helix since its fiber diffraction pattern showed a high similarity of the general view compared with those of native collagens. This is only one structural report other than native collagen which support the Rich and Crick model. In order to clarify this structural discrepancy between (Pro-Pro-Gly)$_n$ (n = 9, 10, and 11) and poly(Pro-Gly-Pro), the structure of poly(Pro-Gly-Pro) should be reanalyzed by using the recent diffraction[24] and structure analysis technique for fibrous polymers.[5]

The ring puckering of the imino acids depends on their position in a Gly-Xaa-Yaa triplet. Based on the two dimensional NMR investigation for the solution structure of PPG10[25] and recent precise analysis of ring puckering,[13] it was reported that proline at Xaa and Yaa position follow a general trend of down-puckering at Xaa and up-puckering at Yaa. This trend was also observed in this study. That is, all the 7 proline residues at Xaa position are down-puckering and 6 proline residues out of 7 at Yaa position are up-puckering.

Lateral Packing of Triple-Helix

The packing structure of triple-helices is essentially the same as that observed in PPG10[6,7] and completely different from that of POG10.[8] There are four symmetry-related triple-helices in a unit cell. As found in PPG10, the packing can be considered to be made up of two different clustering, one triangular and one square columns (Figure 3). In this packing arrangement, four sides of a square are shared with those of surrounding four triangles and three sides of a triangle are shared with those of surrounding two squares and one triangle. This is one of the eleven types of packing patterns[26] for circles with two-dimensional equivalent packing. In the square column, triple-helices placed diagonally run in parallel, and these are antiparallel with those of the other diagonal. Central regions of these triangular and square clusters are expected to be filled with water molecules, some are bound to the pep-

tides directly and some are bound to the other water molecules. Providing that the water content of PPG9 being the same as that of PPG10 (46 wt%),[27] around 80 water molecules are expected in an asymmetric unit. One half of them (34 water molecules) were obtained from difference electron density map and the other half are expected to fill the space between triple-helicies as a free or a partially-free water molecule. Among obtained 34 water molecules, fourteen make direct hydrogen bonds with peptide chains and the rest of them participates in hydrogen bond only with other water molecules. The number of water molecules obtained in this study is about twice of PPG10-1 (15 water molecules). One of the reasons for this may be attributed to the higher resolution limit of PPG9 (1.0 Å) compared with PPG10-1 (1.9 Å). However, it is still less than that of PPG10-2 (40 water molecules and 1.6 Å resolution), which may be attributed to whether *freeR* was used (PPG9) or not used (PPG10-2) during the assignment of water molecules. On the other hand, the number of water molecules obtained here is quite compatible with that found in the recent analysis of PPG10 crystallized under microgravity and with 1.3 Å resolution,[13] in which 36 water molecules were found in an asymmetric unit. Twelve water molecules in an asymmetric unit of PPG9 were found to be essentially in the same position as in the PPG10-1 and PPG10-2 structures.

Longitudinal Packing of Triple-Helix

In X-Ray diffraction patterns of (Pro-Pro-Gly)$_n$ (n = 9, 10[6], 11,[28]), (Pro-Hyp-Gly)$_n$ (n = 10[6], 11[28]), (Pro-Pro-Gly)$_4$-(Ala-Pro-Gly)-(Pro-Pro-Gly)$_4$[29] (hereafter APG-peptide) and (Pro-Pro-Gly)$_4$-(Pro-Ala-Gly)-(Pro-Pro-Gly)$_4$[29] (hereafter PAG-peptide), strong reflections were found on the layer lines corresponding to the 20 Å axial repeat along the c-axis (Figure1). This c = 20 Å repeat was based on the helical symmetry of triple strands as we can see in the radial projection (Figure 4). That is, according to the Okuyama model[2] for collagen, three (right-handed) 7/1-helices with a 60 Å pitch length assemble around the common axis and make a triple-helix (Figure 4a). Here, an ellipse with integral number denotes one triplet, Gly-Xaa-Yaa. In the radial projection (Figure 4b), one peptide chain was shown by a series of ellipses connected by the solid line. This radial projection clearly shows that the fiber period decreases to one third of the helical pitch because of the symmetry among these three chains. Here, if we trace the scattering units (ellipses) along the broken line, these make a left-handed 7/2-helix with a 20 Å axial repeat, as a whole. Since the actual molecular lengths of these peptides are 77 Å for PPG9, PAG-, and

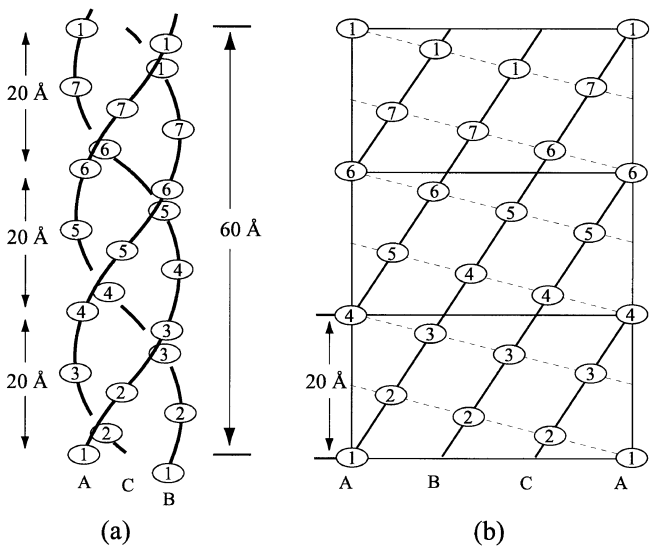

Figure 4. (a) Schematic illustration of the 7/2-helix model. Each strand has a 7/1-helical symmetry within a 60 Å axial repeat. (b) Radial projection of the 7/2-helix. The ellipse with integer in (a) and (b) denotes one triplet, Gly-Pro-Pro.

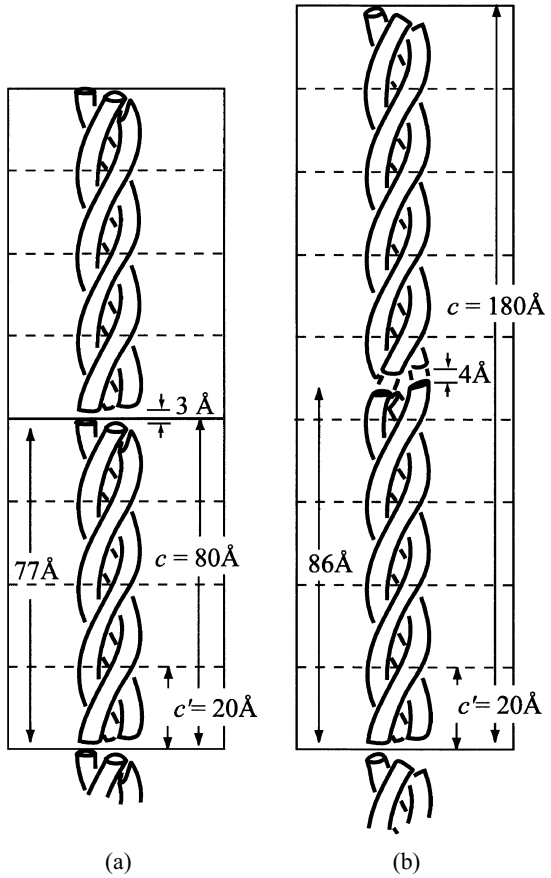

Figure 5. Schematic illustrations of (a) PPG9 and (b) PPG10 and POG10 triple-helical molecules along the c-axis.

APG-peptides, 86 Å for PPG10 and POG10, and 95 Å for PPG11 and POG11, the c-repeats of these peptide crystals should be longer than the corresponding molecular length providing there is no crystallographic sym-

metry to decrease *c*-repeat like in the case of EKG-peptide. From the detailed investigation of satellite spots in the diffraction patterns of PPG10, the longer axial repeat was found to be 180 Å (= 9×20 Å).[12] From precise examination of enlarged diffraction patterns of PPG10 and POG10, we also found the longer axial repeat of these crystals to be 180 Å instead of 100 Å which we accepted in the earlier studies. In the cases of PPG9, PAG- and APG-peptides, their diffraction patterns showed clearly that the longer axial repeat was 80 Å (= 4 × 20 Å). In the cases of PPG11 and POG11, we obtained 100 Å (= 5 × 20 Å) as a long axial repeat. From these experimental results together with crystal structures analyzed so far, we may deduce the longitudinal packing principle for $(Pro\text{-}Pro\text{-}Gly)_n$ and $(Pro\text{-}Hyp\text{-}Gly)_n$. That is, rod-like triple-helices pack longitudinally in a head-to-tail fashion with some gap between adjacent triplexes, so that the repeating period along the *c*-axis is an integral multiple of the strong 20 Å helical repeat (Figure 5). The length of the gap, *i.e.*, the distance between adjacent triplexes, is approximately the sum of van der Waals distances. Since the number of satellite reflections observed in PPG9 is much more than those of PPG10, PPG11, POG10, and POG11, the structure analysis for the c = 80 Å cell is now in progress.

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