Helical twists of collagen model peptides

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Helical Twists of Collagen Model Peptides

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Abstract: *Average helical twists were calculated by the method of Sugeta and Miyazawa (Biopolymers 1967, 5, 673–679) for all of the collagen model peptides analyzed to date. Calculation of the helical twists of all triplets in each peptide strand provided novel insights for several model peptides. In the (Pro-Pro-Gly)*n (n = *9 and 10), the helical twists showed cyclic fluctuations between 40 and 65° with a 20 Å period, suggesting that their molecular conformations were close enough to the ideal 7/2-helix to show the helical repeat of 20 Å. Rather small helical twists in the guest regions of IBP in complex and T3-785 were attributed to the interaction with Integrin I domain and a relaxed conformation caused by three consecutive triplets lacking imino acid residues, respectively. Although most of the triplets used in this study were imino acid–rich triplets, helical twists were scattered in a wide range from 30 to 70° with an overall average of 52.6°. This distribution of helical twists indicated a strong preference for the 7/2-helical conformation (51.4°) rather than the 10/3-helical model (36°).* © 2006 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 84: 421–432, 2006

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INTRODUCTION

Fibrous macromolecules such as DNA, collagen, silk fibroin, etc, take on various characteristic helical conformations. The nature of a helical conformation is described by the helical parameters, unit height (h), defined by an axial rise along the helical axis, and unit twist (θ), defined by a rotation angle around this axis (Figure 1). The helical parameters can be experimentally obtained from X-ray diffraction patterns of oriented fiber specimens by measuring the fiber period from layer line spacing and determining helical symmetry from the intensity distribution on these layer lines. The helical parameters of fibrous specimens provide the most important structural information since they provide a framework for the molecular structure. Therefore, we must determine these parameters before undertaking detailed analysis of the molecular structure. Having the helical symmetry and axial repeat, we can vary dihedral angles in a helical asymmetric unit to refine the molecular conformation to fit the X-ray diffraction data. If we are unable to experimentally determine the unit twist and unit height, we are unable to begin the structural analysis of a polymer.

Unfortunately, in the case of native collagen, the fiber diffraction pattern does not contain enough data to determine the helical parameters definitively. Therefore, two structural models have been proposed with different helical symmetries and axial repeats. These are the left-handed 7/2-helical model, which has a 20 Å axial repeat,[1,2] and the left-handed 10/3-helical model, which has a 28.6 Å axial repeat.[3–5] As shown in Figure 2, each peptide strand of the 7/2 model forms a 7/1-helix with a 60 Å axial repeat and has one triplet as a helical repeating unit. Therefore, the unit twist (θ) and unit height (h) of the peptide strand are 51.4° (360°/7) and 8.57 Å (60 Å/7), respectively. For the 10/3 model, these values are 36° (360°/10) and 8.58 Å (85.8 Å/10). Here, the essential difference between the two models resides not in the unit height, but in the unit twist. The historical background of these two models has been only sparingly cited in the literature.[6] Recently, we refined both structures using continuous X-ray diffraction data from native collagen under the corresponding helical constraints and concluded that the X-ray diffraction data can be explained not only by the prevailing 10/3-helical model but also by the 7/2-helical model.[6]

During the last decade, more than 20 single crystal structures of collagen-model peptides have been reported at high resolution.[7–26] In these analyses, the atomic coordinates of peptide atoms were obtained using the intensity data of several thousand to several tens of thousands of reflections without applying any restraining or constraining conditions on helical symmetry. In fiber diffraction analyses, as discussed above, helical parameters must be obtained before the analysis. On the contrary, in single crystal analyses, the helical parameters can be calculated using the atomic coordinates of the peptide main chain obtained from the structure analysis. Since each chemical repeating unit, a Gly-*X*-*Y* triplet, of each strand in the molecule provides helical parameters, we can understand the local conformation of the molecule in terms of the helical twist (unit twist). Most of the peptides analyzed to date have been rich in imino acid residues to maintain the triple-helical conformation and have shown mainly 7/2-helical symmetry. However, some peptides had varied helical conformations within the same molecule depending on the amino acid sequence. For example, it was reported that the terminal zones of the (Pro-Hyp-Gly)$_3$-Ile-Thr-Gly-Ala-Arg-Gly-Leu-Ala-Gly-(Pro-Hyp-Gly)$_4$ (hereafter, T3-785) peptide maintain approximate 7/2-helical symmetry, whereas the central zone more closely matches 10/3-

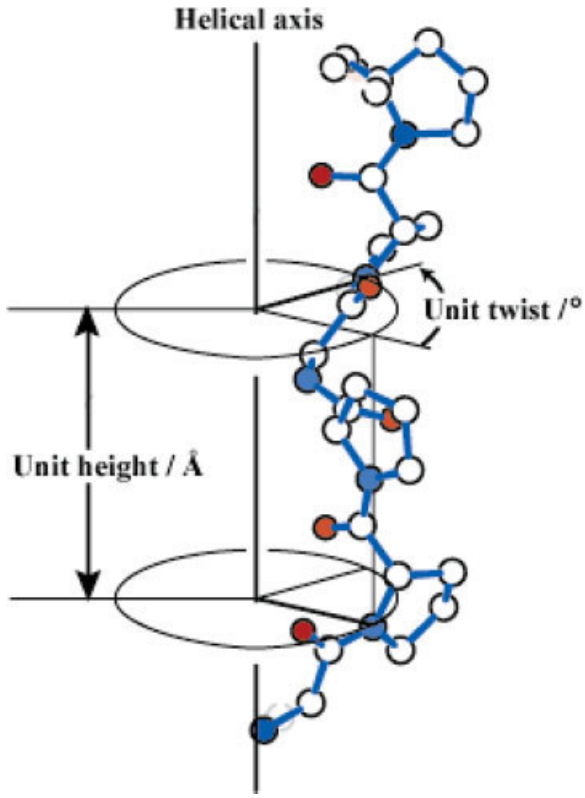

FIGURE 1 Schematic illustration of the helical parameters, unit twist and unit height. When molecules have ideal helical symmetry, these parameters are obtained by measuring the rotation angle around the helical axis and the axial rise along the helical axis between the same atoms in consecutive helical units.

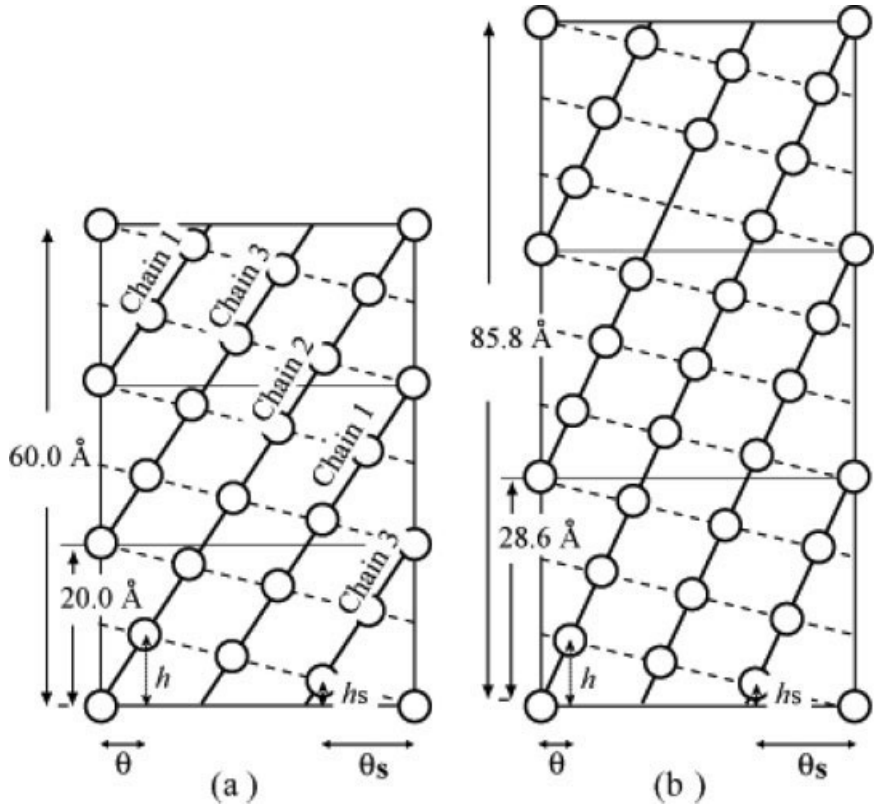

FIGURE 2 Radial projections of the 7/2-helical model (a) and the 10/3-helical model (b) for the molecular structure of collagen. Circles represent one triplet, Gly-*X*-*Y*. Peptide strands are shown by circles connected with solid lines. Chain numbers in (a) correspond to those in Figure 3. (h,θ) and (hs,θs) correspond to the unit height and unit twist of a peptide strand and a superhelix.

helical symmetry.[12,16] For the (Gly-Pro-Hyp)$_2$-Gly-Phe-Hyp-Gly-Glu-Arg-(Gly-Pro-Hyp)$_3$ peptide (hereafter, IBP) in complex with the integrin I domain, it was reported that the terminal regions approximate 7/2-helical symmetry, whereas the central GFOGER region approximates 10/3-helical symmetry.[13] In the former case,[12,16] the helical twist of each amino acid residue was obtained by measuring the rotation angle between adjacent triplets within the same strand. Although the twist angles in the whole molecule obtained by this method showed a similar tendency as those calculated in this study, the absolute values of each triplet were different. In the latter case,[13] the local conformation was compared with each of the two ideal helical models by superposition and root mean square deviation. Furthermore, some of the helical twists cited in the most recent review article[27] are very different from those calculated by our method.[25] That is, they calculated unit twists for super helices, and their values were very different from ours. For example, the unit twist of the (Pro-Pro-Gly)$_{10}$ (hereafter PPG10) and (Pro-Pro-Gly)$_9$ (PPG9) superhelices were noted to be -104.3 and -102.9° (Table II of ref. 27), respectively. In contrast, the helical twists for these two molecules obtained by our method are essentially identical, 51.6° for PPG10_B and 51.8° for PPG9_H (Table I). A difference of 1.4° between helical twists of super helices is quite large considering that the unit twists of the ideal 7/2- and 10/3-helices are -102.9 and -108°, respectively. Furthermore, the root mean square deviation (rmsd) of the backbone atoms in the 10/3-helical model proposed by Ramachandran and the model proposed by Rich and Crick is more than twice that of the rmsd of the Ramachandran model and the 7/2-helical conformation observed in PPG9 (Table I of ref. 27).

The above reports confused our understanding of the helicity of the triple-helix and prompted us to recalculate the helical parameters of all of the collagen-model peptides reported to date using the method of Sugeta and Miyazawa.[28] Since this method follows the mathematical relationship between the helical parameters and the intramolecular coordinates of the peptide main chain, we believe that it is the most appropriate and convenient method for calculating the helical parameters. Using this method, the helical

Table I Helical Twists of Collagen Model Peptides

Peptide	PDB Code	Temperature (K)	Resolution (Å)	Helical Twist Host (°)	Helical Twist Guest (°)	Reference Number
PPG*n* sub cell			**(Pro-Pro-Gly)*n***			
PPG9_H	1TT	RT	1.1	51.6		18
PPG10_N		RT	1.9	52.0		10
PPG10_K1	1A3I	259	1.97	52.0		9
PPG10_K2	1A3J	293	1.6	51.5		9
PPG10_V	1G9W	RT	1.3	51.5		15
PPG*n* full-cell			**(Pro-Pro-Gly)*n***			
PPG9_H	2CUO	100	1.33	51.8		25
PPG10_B	1K6F	RT	1.3	51.6		19
POG*n* sub cell			**(Pro-Hyp-Gly)*n***			
POG10_N		RT	1.9	51.9		11
POG10_B		100	1.4	—		17
POG10_O	1V7H	100	1.25	51.9		21
POG11_O	1V4F	100	1.26	51.9		21
POG11_O	1V6Q	RT	1.25	53.7		21
GOO9			**$(Gly\text{-}Hyp\text{-}Hyp)_9$**			
	1YM8	100	1.55	51.7		24
GPP10-foldon			**Gly-Ser-$(Gly\text{-}Pro\text{-}Pro)_{10}$-foldon**			
	1NAY	100	2.6	51.5		20
PPG9-PAG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Pro-Ala-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
		100	1.35	53.2	47.0	23
		RT	1.15	52.7	50.0	
PPG9-AAG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Ala-Ala-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
		100	1.39	51.8	50.5	
PPG9-POG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Pro-Hyp-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
	2D3F	100		51.4	55.1	
PPG9-PaOG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Pro-alloHyp-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
	1X1K	100	1.1	51.4	51.9	26, 39
PPG9-OPG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Hyp-Pro-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
		100	1.36	52.1	52.1	
PPG9-OTG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Hyp-Thr-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
		100	1.3	53.0	48.9	
PPG9-OOG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Hyp-Hyp-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
	2D3H	100	1.22	51.7	52.0	
PPG9-ODG			**$(Pro\text{-}Pro\text{-}Gly)_4$-Hyp-Asp-Gly-$(Pro\text{-}Pro\text{-}Gly)_4$**			
		100	1.02	49.0	52.6	
POG10-LOG1			**$(Pro\text{-}Hyp\text{-}Gly)_4$-Leu-Hyp-Gly-$(Pro\text{-}Hyp\text{-}Gly)_5$**			
		100	1.6	54.3	51.1	23
POG10-LOG2			**$(Pro\text{-}Hyp\text{-}Gly)_4$-$(Leu\text{-}Hyp\text{-}Gly)_2$-$(Pro\text{-}Hyp\text{-}Gly)_4$**			
		95	1.4	53.6	46.9	
EKG peptide			**$(Pro\text{-}Hyp\text{-}Gly)_4$-Glu-Lys-Gly-$(Pro\text{-}Hyp\text{-}Gly)_5$**			
	1QSU	277	1.75	51.8	46.8	14
POG8-PAG			**$(Pro\text{-}Hyp\text{-}Gly)_3$-Pro-Ala-Gly-$(Pro\text{-}Hyp\text{-}Gly)_4$**			
		95	1.2	52.6	48.1	
POG8-PRG			**$(Pro\text{-}Hyp\text{-}Gly)_3$-Pro-Arg-Gly-$(Pro\text{-}Hyp\text{-}Gly)_4$**			
		100	1.45	53.9	48.4	
(Gly → Ala) peptide			**$(Pro\text{-}Hyp\text{-}Gly)_4$-Pro-Hyp-Ala-$(Pro\text{-}Hyp\text{-}Gly)_5$**			
	1CAG	263	1.85	58.3	36.8	7
	1CGD	263	1.85	57.3	36.1	8

Table I (*Continued from the previous page.*)

Peptide	PDB Code	Temperature (K)	Resolution (Å)	Helical Twist (°) Host	Guest	Reference Number
PG peptide			**(Pro-Hyp-Gly)$_4$-Pro-Gly-(Pro-Hyp-Gly)$_3$**			
	1EI8	123	2.0	54.7	−60.2[a]	
IBP			**(Gly-Pro-Hyp)$_2$-Gly-Phe-Hyp-Gly-Glu-Arg-(Gly-Pro-Hyp)$_3$**			
	1Q7D	85	1.8	50.0	44.4	22
A-chain				52.1	42.5	
B-chain				48.6	44.8	
C-chain				49.0	45.9	
IBP in complex			**(Gly-Pro-Hyp)$_2$-Gly-Phe-Hyp-Gly-Glu-Arg-(Gly-Pro-Hyp)$_3$**			
	1DZI	100	2.1	50.6	44.5	13
A-chain				50.3	40.3	
B-chain				51.9	41.0	
C-chain				49.6	52.4	
T3-785 peptide			**(Pro-Hyp-Gly)$_3$-Ile-Thr-Gly-Ala-Arg-Gly-Leu-Ala-Gly-(Pro-Hyp-Gly)$_4$**			
	1BKV	123	2.0	52.4	41.8	16
A-chain				52.0	40.6	
B-chain				55.0	41.6	
C-chain				50.5	43.3	

[a] A detailed explanation is provided in the text and in Table 2.

parameters of each repeating unit were calculated in structural studies of the α-helix and 3_{10} helix of oligopeptides, where helical parameters aided the understanding of local conformation.[29–32] Since the triple-helical conformation of collagen-model peptides is much more complicated than a simple helix of one strand having one amino acid residue as a helical repeating unit, utilization of helical parameters to understand local conformation of a triple-helix is much more effective than that for a simple helix. In this study, we introduce the method of Sugeta and Miyazawa[28] to calculate helical twist from atomic coordinates obtained from single crystal analyses of collagen model peptides and present novel findings from these calculations.

METHODS

Definition of Helical Parameters

The helical parameters, unit twist (or helical twist) and unit height, describe the spatial arrangement of repeating units of helical polymers. Unit twist is defined by a rotation angle (θ) around the helical axis and unit height by an axial translation (h) along the helical axis per one helical repeating unit (Figure 1). When the molecule has m/n-helical symmetry and a fiber period of c Å, the unit twist and unit height will be ($360^\circ \times n/m$) and (c/m), respectively, where m and n represent the number of helical repeating units and the number of helical turns, respectively. The unit twist and unit height are also shown in radial projections of two structural models of collagen (Figure 2). The helical twists of each peptide strand of the ideal 7/2- and 10/3-helical models are 51.4° (360°/7) and 36° (360°/10), respectively, since in these models each peptide strand has 7/1- and 10/1-helical symmetry as shown in Figure 2. In some literature, the unit twist of the ''superhelix'' is also mentioned. The unit twist of the superhelix is obtained by considering the hypothetical single helix with 7/2-helical symmetry or 10/3-helical symmetry. These hypothetical single helices can be traced by the broken lines while the three actual peptide helices are traced by solid lines (Figure 2). The unit twist of the hypothetical superhelix, θs, is related to the unit twist, θ, of the actual helix by the following equation:

$$\theta_s = -n \times \theta \quad (n = 2 \text{ for the } 7/2\text{- and 3 for the } 10/3\text{-helical model}) \tag{1}$$

Therefore, when we consider an ideal helix, the designation of one type of helical twist is more than enough to show the helical features of the polymer molecule. However, in the case of collagen model peptides, the helical parameters of one triplet are calculated from the atomic coordinates of the peptide main chain in the corresponding triplet according to the mathematical relationship described in the next section. Therefore, the obtained helical twist for the peptide strand assumes that the conformation of the examined triplet repeats infinitely. To obtain the unit twist of a superhelix, we have to focus on the relationship between two triplets. In fact, the two triplets usually have slightly different con-

formations. There are many ways to calculate the unit twist of a superhelix; this has led to discrepancies between different research groups. Furthermore, there is no simple relationship between the unit twist of an actual peptide strand and the hypothetical superhelix since Eq. (1) is valid only in the case of an ideal helix. When we discuss the ideal triple-helix, it is very convenient to presume the triple-helical molecule to be a hypothetical single helix. Therefore, we usually use the 7/2- (or 10/3-) helix instead of three peptide strands with 7/1- (or 10/1-) helical symmetry. However, for molecular conformations obtained by single crystal analyses, we calculated the helical parameters for each triplet in each peptide strand.

General Method

In the secondary structure of proteins, such as α-helix, 3_{10}-helix, ω-helix, and β-sheet, the helical repeating unit consists of one amino acid residue that contains three main chain atoms: N, Cα, and C′. The method of Sugeta and Miyazawa[28] to calculate helical parameters requires nine input parameters for each amino acid residue. These are three bond lengths, three bond angles, and three dihedral angles, as shown below for the *j*th amino acid.

$$\text{Bond N}(j)-\text{C}\alpha(j) \quad \text{Angle C}'(j-1)-\text{N}(j)-\text{C}\alpha(j)$$
$$\phi\ \text{C}'(j-1)-\text{N}(j)-\text{C}\ \alpha(j)-\text{C}'(j)$$

$$\text{Bond C}\alpha(j)-\text{C}'(j) \quad \text{Angle N}(j)-\text{C}\alpha(j)-\text{C}'(j)$$
$$\psi\ \text{N}(j)-\text{C}\alpha(j)-C'(j)-\text{N}(j+1)$$

$$\text{Bond C}'(j)-\text{N}(j+1) \quad \text{Angle C}\alpha(j)-\text{C}'(j)-\text{N}(j+1)$$
$$\omega\ \text{C}\ \alpha(j)-\text{C}'(j)-\text{N}(j+1)-\text{C}\ \alpha(j+1)$$

Since one helical repeating unit of collagen-helix consists of three amino acid residues, three consecutive sets of parameters must be prepared before the calculation. That is, the helical parameters of the *j*th triplet were calculated from three sets of nine parameters for the *j*th, (*j*+1)th, and (*j*+2)th residues. Therefore, in host–guest peptides in which the guest amino acid starts from the *j*th residue, the helical parameters of the (*j*–2)th and (*j*–1)th triplets may be influenced by the guest amino acid. Three peptide strands in the same molecule are staggered along the helical axis by one amino acid residue. Furthermore, the conformational change of one chain affects the other two chains in the same molecule. Because of this, in some cases, we observed unit twist values that deviated from the average not only in the guest peptide but also near the guest peptide.

Calculation Procedure

Bond lengths, bond angles, and dihedral angles were calculated using the SHELXL[33] program and atomic coordinates obtained from the Protein Data Bank (PDB). The list of collagen-model peptides reported to date is shown in Table I, together with the PDB codes and abbreviation used in this study. Some host–guest peptides, which we have already analyzed but have not yet reported, are also included in Table I. Helical parameters were calculated using an in-house program, PHEL. The desired bond lengths, bond angles, and dihedral angles were picked up from the list file (***.lst) of SHELXL, and rearranged so as to fit the input data for PHEL by the in-house L2H program. Here, the input data for the helical parameters of the *j*th triplet consist of three sets of nine parameters for the *j*th, (*j*+1)th, and (*j*+2)th amino acid residues. Helical parameters were calculated for every amino acid residue.

Since the conformations in the terminal region have a large degree of freedom, and hence deviate greatly from those in the rest of the molecule in some cases, these unit twists were not included in the average calculation. In the cases of (Pro-Pro-Gly)$_n$ (hereafter, PPG*n* where $n = 9$ or 10) and (Pro-Hyp-Gly)$_n$ (POG*n* where $n = 10$ or 11), subcell structures within a 20 Å axial repeat were analyzed assuming that the molecules were infinite since there were not sufficient reflection data other than those on the layer lines corresponding to the 20 Å axial repeat. Since there is no molecular terminus in these cases, there was no terminal effect, as was usually observed in other cases.

RESULTS AND DISCUSSION

Peptides with Imino Acid Residues in Both the X and Y Positions

The average unit twists of PPG*n* ($n = 9$ or 10) in both sub- and full-cell structures, and POG*n* ($n = 10$ or 11) in subcell structures showed very similar values that were close to, but slightly larger than the unit twist of an ideal 7/2-helix (51.4°). The average unit twist seems to be independent of the resolution of analysis, data measurement temperature, the difference of subcell and full-cell structures, unit cell dimensions, and packing arrangement (Table I). The average unit twist of (Gly-Hyp-Hyp)$_9$ (hereafter, GOO9) showed the same tendency despite rather different (ϕ,ψ) values of Hyp at the *Y* position (-66.2°, 156.4°) compared with related model peptides.[24] The GPP10-foldon is very different from the other peptides in Table I since this peptide consists of both globular and triple-helical (Gly-Pro-Pro)$_{10}$ parts. Because of this unique molecular conformation, the packing arrangement of this peptide is different from other peptides with the same Gly-Pro-Pro sequence. Namely, one GPP10-foldon molecule is surrounded by four antiparallel molecules, while one PPG*n* molecule is surrounded by five adjacent molecules, one of which is parallel and the other four antiparallel. Despite these differences, the unit twist (51.5°) of the triple-helix portion of GPP10-foldon is quite similar to the unit twists of other studied peptides and the

ideal 7/2-helix. Although the average unit twists of PPG*n* and POG*n* are close to the unit twist of the ideal 7/2-helix, the value of each unit twist is scattered in a very wide region from 40 to 60°. The specific unit twist of each amino acid residue is discussed in the next section.

The only exception in this series of peptides is poly(Pro-Gly-Pro) analyzed using a fiber diffraction technique.[34] Although this polymer has imino acid residues in both the *X* and *Y* positions, it was reported that the polymer molecule has 10/3-helical symmetry ($\theta = 36°$) based on a fiber diffraction pattern similar to that of native collagen. In this context, the Rich and Crick model for the molecular structure of collagen (the 10/3-helical model) is very interesting since they built their model based on the Gly-Pro-Hyp sequence.[5] According to the recent single crystal analyses as shown in Table I, peptides rich in imino acid residues showed absolutely 7/2-helical symmetry.

Cyclic Fluctuation of Unit Twists Observed in PPG9 and PPG10

The unit twists of each amino acid residue in the three peptide strands of PPG9 are plotted in Figure 3. Molecules A and B in an asymmetric unit are plotted separately. Two features are observed in this figure. First, there is a fairly large fluctuation of the unit twist, which ranges from 40 to 65° (average 51.8°). This amount of deviation from the average value was usually observed in other collagen-model peptides, including host–guest peptides. Second, this fluctuation is cyclic. The period of the cyclic fluctuation corresponds to the helical repeating unit, 20 Å. During the refinement calculation, no restriction that the peptide molecules must take a helical conformation was applied. Therefore, these peptides took on a 20 Å repeating period of their own accord. If molecules have ideal 7/2-helical symmetry in an isolated state, all of the triplets in the three peptide strands take on the same value of unit twist (51.4°), as shown by the broken line in Figure 3. In the crystal state, however, the detailed conformation of these molecules is influenced by the surrounding molecules; hence, the unit twist of each triplet deviates from the ideal and the conformation shows only a pseudo-fiber repeating period of 20 Å. This pseudo-fiber period appears in the structures because of the consecutive head-to-tail association of triple-helices along the helical axis. According to the radial projection of the ideal 7/2-helix (Figure 2a), one triplet in chain 2 coincides with the triplet of chain 3 after a translation of 20 Å along the helical axis. This relationship is also seen in Figure 3. For example, in the A molecule, the unit twist of Res_205 in chain 2 is located at the local minimum of this chain, while the unit twists of Res_314 in chain 3 and Res_120 in chain 1 are also located at the local minima of their corresponding chains. These three residues in the different chains are located at the same position 20 Å apart from each other along the helical axis and have similar interactions with adjacent molecules (Figure 4).[25] The strong pseudo-fiber period of 20 Å found in the cyclic fluctuation of helical twists made the full-cell structure analyses of PPG9 very difficult. This type of cyclic fluctuation is also observed in PPG10, PPG9-PAG, PPG9-POG, and PPG9-OPG. The packing structures of these peptides are very similar to each other.

Host–Guest Peptides

It is usually difficult for (Gly-*X*-*Y*)$_n$ peptides to form a triple-helical structure unless both the *X* and *Y* positions are occupied by imino acid residues. Therefore, when we need physicochemical information about such a sequence, we use host–guest peptides in which guest triplets are sandwiched by host peptides that aid stable triple-helix formation.[35,36] Most of the peptides in Table I belong to this class. Among these, we shall consider the (Gly→Ala) peptide, the PG peptide, IBP, and T3-785 in a separate section since there are many interesting points in these studies. The remainder of the host–guest peptides have one or two guest triplets embedded in the central region of the host PPG9, POG10, or POG8 peptides. Various types of guest peptides were examined. Some are frequently observed triplets in the collagen sequence, such as Pro-Ala-Gly, Ala-Ala-Gly, Pro-Hyp-Gly, Glu-Lys-Gly, Pro-Arg-Gly, and Leu-Hyp-Gly. Some are artificial triplets, such as Pro-alloHyp-Gly, Hyp-Pro-Gly, Hyp-Thr-Gly, Hyp-Hyp-Gly, and Hyp-Asp-Gly. Most of the average unit twists of the guest regions were similar to those of the host regions. Slightly smaller values were observed in the PPG9-PAG, PPG9-AAG, POG8-PAG, EKG peptide, and POG10-LOG2 structures. This evidence indicates that it is difficult to affect the local conformation of only one guest triplet embedded in an imino acid–rich sequence. Slightly larger values were observed in the PPG9-POG, PPG9-PaOG, PPG9-OPG, and PPG9-OOG structures, in which the guest peptide has imino acid residues in the *X* and *Y* positions.

(Gly→Ala) Peptide

The (Gly→Ala) peptide is the first collagen model peptide analyzed by protein crystallography. Although the glycine residue at every third position is the most

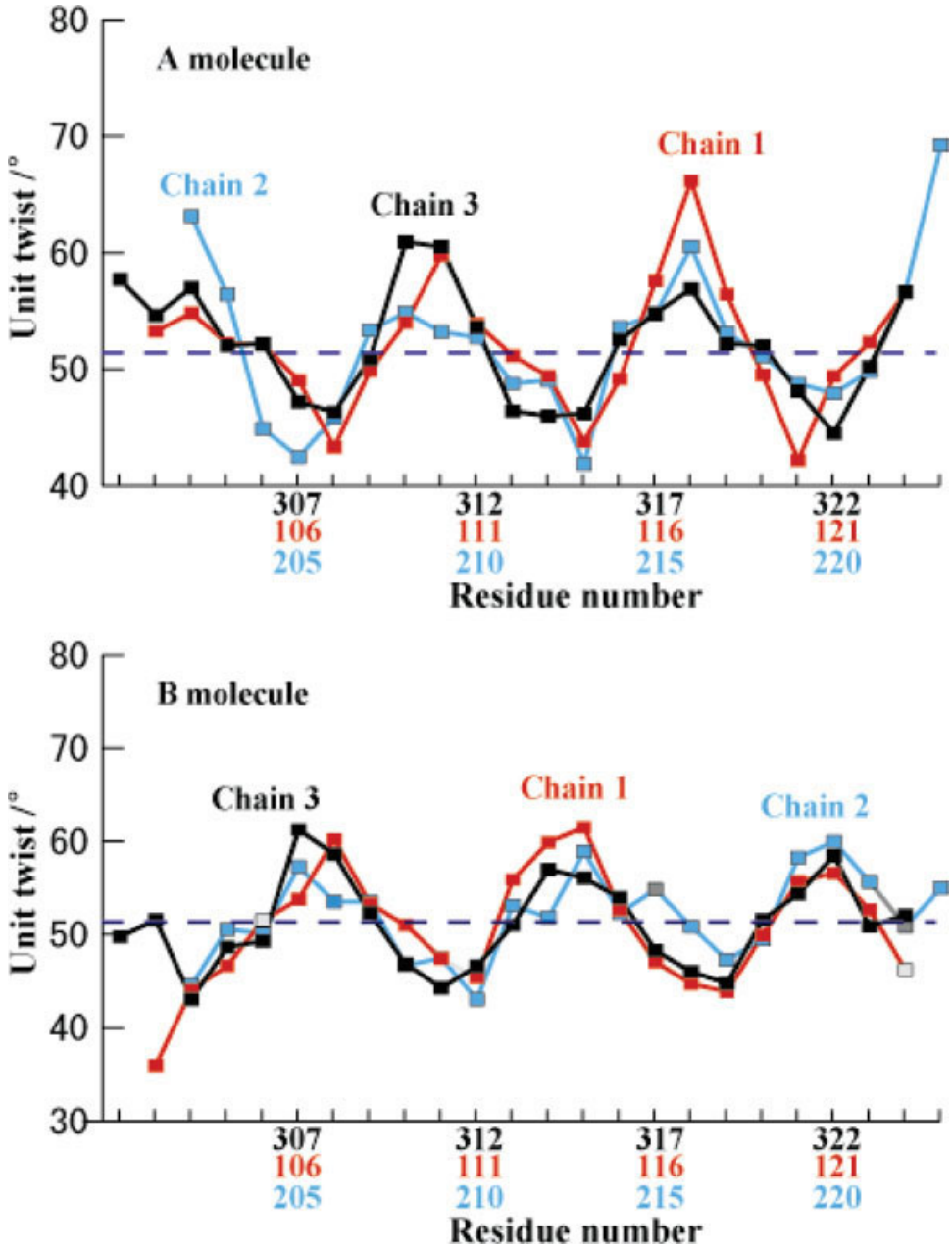

FIGURE 3 Cyclic fluctuation of unit twists observed in $(Pro\text{-}Pro\text{-}Gly)_9$. Unit twists of different chains are shown in different colors. There is one residue of stagger between chains 3 and 1 and chains 1 and 2, as shown in Figure 2.

important feature of the collagen primary structure, the (Gly→Ala) peptide has substituted Ala for Gly in the central triplet of the POG10 sequence. This substitution interrupts stable triple-helix formation and is related to the fatal disease osteogenesis imperfecta.[7] In fact, the average unit twist of the guest region (36.8° for 1CAG and 36.1° for 1CGD) is very loose compared with that of the host region (58.3° for 1CAG and 57.3° for 1CGD) to avoid steric compression around the Ala residue. Although the unit twist of the guest region is very close to that (36°) of the ideal 10/3-helix, this has no meaning because of the irregular guest sequence. Line graphs of unit twists in the three peptide strands are shown in Figure 2 of reference 7. Although the tendency of twist angles is very similar to our data, the detailed values are rather different. For example, we observed two unit twists (27.4° and 29.5°) less than 30°, while four unit twists less than 30° and two unit twists less than 10° are shown in Figure 2 of reference 7. In many papers,

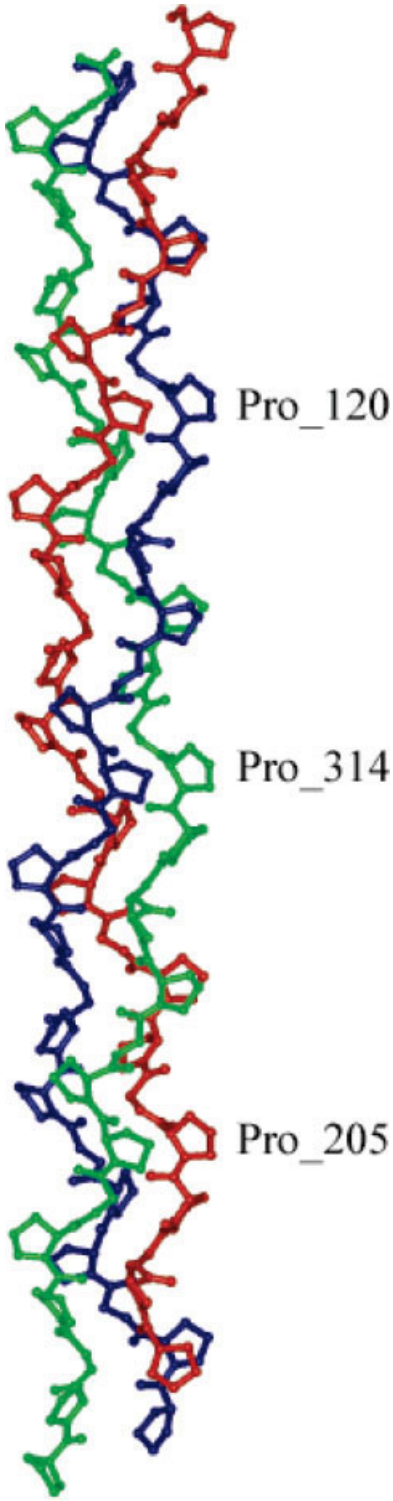

FIGURE 4 Molecular structure of $(Pro\text{-}Pro\text{-}Gly)_9$ showing three proline residues related by a pseudo 20 Å translation.

helical twists obtained from structure analyses have been compared with those found in other peptides together with those of the ideal 7/2- and 10/3-helices. However, such comparison is meaningful only when those values are calculated based on the same reproducible method.

PG Peptide

The PG peptide lacks one hydroxyproline residue in the *Y* position of the central triplet in the POG8 peptide and shows a very clear difference in unit twist between the host and guest regions. In the host region, the average unit twist is close to that of the ideal 7/2-helix; however, in the guest region, the sense of the helix is opposite ($\theta = -60.2^\circ$) (Table I). Chemically, the guest region of the PG peptide consists of two amino acid residues, Pro_13 and Gly_14. Therefore, in this peptide, the average unit twist of the guest region cannot be calculated. An asymmetric unit of the PG peptide contains two molecules, A and B. Chain 1 in the B-molecule has unusual unit twists from residues 12 to 14, while unusual unit twists are observed in residues 11 to 13 of chain 2 and residues 10 to 12 of chain 3, as shown in Table II. Therefore, residue numbers with unusual unit twists are shifted by one residue between chains 1 and 2, and chains 2 and 3. Since there is one residue of stagger between adjacent strands in the same molecule, residues with unusual unit twists are located in the same area along the helical axis. The main contribution to the unusual unit twists likely derives from the unusual ϕ and ψ values of different residues in each strand. That is, Gly_14 in chain 1 ($\phi,\psi = -109.4^\circ,84.5^\circ$), Pro_13 in chain 2 ($\phi,\psi = -99.2^\circ,75.7^\circ$), and Gly_12 in chain 3 ($\phi,\psi = -140.1^\circ,73.3^\circ$). A similar situation was also observed in molecule A. In this case, unusual ϕ and ψ values were found in Gly_14 and Pro_13 of chain 1 ($\phi,\psi = 96.1^\circ,106.4^\circ$) and ($\phi,\psi = -58.0^\circ, -89.0^\circ$), respectively; Gly_12 of chain 2 ($\phi,\psi = -115.9^\circ$, 78.9°); and Hyp_11 in chain 3 ($\phi,\psi = -107.0^\circ$, 74.3°). In these regions, both molecules rewind from right-handed to left-handed since the average unit twists of the host and guest regions are 54.7° and -60.2°, respectively.

Isolated IBP and IBP in Complex with Integrin

IBP is a very interesting peptide, as the structure of IBP has been solved in both the isolated state[22] and in complex with integrin.[13] Therefore, we can study the conformational changes between isolated IBP and the IBP–integrin complex. Unfortunately, comparison of helical twists was performed only by calculating rmsd of superposed IBP, IBP in complex, the ideal 7/2-helix, and the ideal 10/3-helix.[22] Therefore, the

Table II Helical Twists of the Central Regions of the Six Peptide Strands of the PG Peptide

	A Molecule			B Molecule		
Residue No.	Chain 1	Chain 2	Chain 3	Chain 1	Chain 2	Chain 3
Hyp_8	54.3	51.3	58.2	53.5	62.7	43.9
Gly_9	59.1	52.8	**−56.0**	53.7	57.6	54.8
Pro_10	48.9	**−72.7**	**−72.3**	68.2	46.0	**−81.6**
Hyp_11	**163.8**	**−66.8**	**−71.4**	56.6	**−50.7**	**−65.6**
Gly_12	**−63.6**	**−50.3**	49.3	**−49.7**	**−62.7**	**−73.2**
Pro_13	**−69.7**	64.7	57.8	**−89.3**	**−66.3**	65.9
Gly_14	**−168.7**	56.0	54.6	**−77.7**	41.8	55.1
Pro_15	55.7	50.1	37.2	39.0	51.9	54.4

insights from these structures have not been clearly elucidated. The authors concluded that the IBP central zone takes on an intermediate helical twist between those of the 7/2- and 10/3-helices,[22] while the central GFOGER sequence of IBP in complex approximates 10/3-helical symmetry.[13,37] However, according to our calculations (Table I), there is no significant difference of the average unit twists of the guest regions in IBP (44.4°) and IBP in complex (44.5°).

In Table I, the average unit twists of the host and guest regions of each peptide strand of IBP and IBP in complex are listed together with the average values of three strands. Although the average unit twists of the three strands of IBP and IBP in complex are very similar, the unit twist of each strand of IBP in complex has an important discrepancy. That is, the unit twists of the guest regions of two strands are about 40°, but the unit twist of the third strand is 52.4°. We found that this unexpected difference of unit twist can be attributed to the interaction between the guest GFOGER peptide and the Integrin I domain. That is, the two GFOGER chains with smaller helical twists interact with the Integrin I domain, whereas the third GFOGER chain with the larger helical twist (52.4°) has no interaction with Integrin I. Therefore, the smaller unit twist of IBP in complex is not due to 10/3-helical symmetry, but rather the interaction with the Integrin I domain.

T3-785 Peptide

The T3-785 peptide contains a guest peptide of 12 residues that is identical to residues 785 to 796 of human type III collagen, capped with $(Pro\text{-}Hyp\text{-}Gly)_3$ ends to aid helical stability.[16] The first three triplets of the guest peptide do not contain imino acid residues in both the *X* and *Y* positions, which allows a relaxed conformation compared with the relatively tight structure in the host peptides. The helical twists of the 33 amino acid residues in the guest region of the three peptide strands range from 30 to 54°, and only 4 unit twists are less than 36°. There is an argument that the average helical twist (52.4°) of the host peptide matches the 7/2-helical model, while that (41.8°) of the guest peptide matches the Rich and Crick model (10/3-helical model).[16] However, if this is so, it is quite unusual as the Rich and Crick model was built based on the peptide with Gly-Pro-Hyp sequence[4,5] and refined for this sequence.[38] That is, this argument states that the Rich and Crick model, built for the Gly-Pro-Hyp sequence, does not agree with the peptide conformation in the imino acid–rich region, but does agree with the conformation in the region lacking imino acid residues. Therefore, it is perhaps more rational to attribute the small average helical twist in the guest region of the T3-785 peptide to the relaxed conformation caused by absence of imino acid residues.

Distribution of Helical Twists Found in Model Peptides

The distribution of helical twists of all triplets (2163) in the model peptides (blue color) is shown in Figure 5. Since the helical twists in its guest region have the opposite helical sense, the PG peptide was not included in this statistic. Furthermore, the subcell structures of PPG*n* ($n = 9$ or 10) and POG*n* ($n = 10$ or 11) are not included in this figure because these molecules were assumed to be infinite and constraining conditions for chain continuity were applied in their analyses. Although most of the helical twists are for imino acid–rich triplets, they showed a wide distribution, scattered from 30 to 75° with an average of 52.6°. Before this study, such a wide range distribution of helical twists was not expected because of the rigid conformation of imino acid residues. Although the number of data is small, the distribution of helical

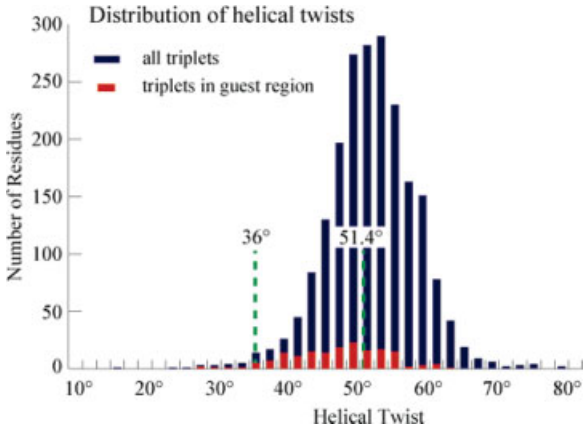

FIGURE 5 Distribution of helical twists for all triplets (blue) and for triplets in the guest region (red). The average helical twist of all triplets is 52.6° and the average helical twist of the guest region is 47.9°. Helical twists for the ideal 7/2- (51.4°) and 10/3-helical models (36°) are marked by green broken lines.

twists for guest regions only (red color) is flat and shifted to the left, with an average helical twist of 47.9°. Both average helical twists are very close to the ideal value of the 7/2-helical model (51.4°) for native collagen.[1,6] On the other hand, there is no indication of even a very small peak around 36°, which corresponds to the ideal twist value of the 10/3-helical model.[3–6] Considering that the Rich and Crick model was built[4,5] and refined[38] for the Gly-Pro-Hyp sequence, there should be some indication of this model in the distribution of helical twists if it indeed represents a stable molecular conformation for collagen. The sequential polymer, poly(Pro-Gly-Pro), was analyzed by Yonath and Traub[34] and shown to form a 10/3-helical structure, which was once regarded as a refined structure of the Rich and Crick model.[40] However, the 10/3-helical symmetry of poly(Pro-Gly-Pro) completely conflicts with the distribution of helical twists observed in this study.

CONCLUSION

In this study, we have calculated the average helical twists of host and guest regions of all collagen-model peptides analyzed at high resolution to date. The average helical twists of the host regions were very close to that of the ideal 7/2-helical model. Except for the unusual (Gly→Ala) and PG peptides, the average helical twists of the guest regions showed similar but slightly smaller values of unit twist compared with the ideal 7/2-helical model. Rather small helical twists observed in the guest regions of IBP in complex and T3-785 were attributed to interactions with the Integrin I domain and a relaxed conformation caused by three consecutive triplets lacking imino acid residues, respectively. The statistics of the distribution of helical twists strongly indicated a preference for the 7/2-helical conformation rather than the now prevailing 10/3-helical model.

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