

X-Ray Structural Studies of Some Poly(α -amino acids) with Hydrophobic Side Chains: Poly(L-valine), Poly(L-isoleucine), and Poly(L-phenylalanine)

Osamu YAMASHITA,*¹ Takashi YAMANE,*¹ Tamaichi ASHIDA,*¹
Shinsuke YAMASHITA,*² and Takuya YAMASHITA*²

*¹Department of Applied Chemistry, Faculty of Engineering, Nagoya University,
Chikusa-ku, Nagoya 464, Japan.

*²Faculty of Pharmaceutical Sciences, Tokushima University,
Tokushima 770, Japan.

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ABSTRACT: X-ray structure studies of poly(L-valine), poly(L-isoleucine), and poly(L-phenylalanine) have been carried out. Poly(L-valine) has two structural forms. One is an α -helical conformation packed in the hexagonal lattice with a dimension of $a=11.43 \text{ \AA}$, though this conformation is unfavorable for poly(L-valine). The other is a β pleated sheet structure with the crystal data: P2₁2₁2₁, $a=4.80 \text{ \AA}$, $b=19.14 \text{ \AA}$, $c=6.59 \text{ \AA}$ (fiber axis). The β poly(L-valine) gave an X-ray fiber photograph of high quality and a detailed structural study was carried out. Poly(L-isoleucine) also crystallizes in a β pleated sheet structure with $a=4.8 \text{ \AA}$, $b=23 \text{ \AA}$, $c=6.6 \text{ \AA}$, orthorhombic. Contraction of fiber axes in both β structures are observed. The X-ray fiber photograph of poly(L-phenylalanine) suggests the coexistence of ω - and α -helix. This specimen may be the third example of an ω -helix. The significance of the hydrophobic interaction of the side chains in the structures is discussed.

KEY WORDS Poly(L-valine) / Poly(L-isoleucine) / Poly(L-phenylalanine) / α -Helix / ω -Helix / β Pleated Sheet / X-Ray Diffraction / Hydrophobic Interaction /

Detailed X-ray investigation of poly(α -amino acids) with hydrophobic side chains have been carried out occasionally, although many spectroscopic studies have been reported. One of the reasons for this may be the difficulty in obtaining high-molecular-weight film specimens which can be stretched to give well oriented fibers. Only the crystal structures of poly(L-alanine)¹ and poly(L-proline)² have been fully studied by the X-ray diffraction technique. Recently, an improvement in the polymerization method has made it possible to prepare high-molecular-weight homopolymers of hydrophobic α -amino acids and obtain film specimens for X-ray diffraction experiments.

It was suggested from the infrared and Raman spectroscopic studies of the film specimens that poly(L-valine) could also exist as an α -helical structure, although a β -sheet structure is the preferred one.³ This suggestion is very interesting, because L-valine, disubstituted on the β -carbon atom, is known

as one of the nonhelix-forming α -amino acids according to Blout *et al.*⁴

In this paper we will describe the conformation and crystal structure of β poly(L-valine), together with the preliminary crystallographic studies of α poly(L-valine), poly(L-isoleucine), and poly(L-phenylalanine).

MATERIALS AND METHODS

Preparation of the Polymers

High molecular weight samples of poly(L-valine), poly(L-isoleucine), and poly(L-phenylalanine) were prepared by polymerizing the *N*-carboxyanhydrides of corresponding amino acids in anisole.³

Preparation of Films and Oriented Fiber Specimens

The α -form specimens of poly(L-valine) were obtained by isolating thin films from the polymerization medium. The β -form specimens of poly(L-

valine), and the specimens of poly(L-isoleucine) and poly(L-phenylalanine) were obtained by the following method: High-molecular-weight polymers were first dissolved in trifluoroacetic acid (TFA), and cast into films of a few mm thickness. Next cast films of poly(L-valine) and poly(L-isoleucine) were swollen with 8.0% dichloroacetic acid (DCA) in carbon tetrachloride (CCl_4), and then stretched. The remaining solvent was removed by heating. Fiber specimens of poly(L-phenylalanine) were obtained by stretching in toluene. The stretching ratios were 5–10 for poly(L-valine), about 3 for poly(L-isoleucine), and about 5 for poly(L-phenylalanine).

X-Ray Diffraction Measurement

X-ray diffraction photographs were taken with an evacuated cylindrical camera with a diameter of 7 cm, and a flat plate camera using Ni-filtered $\text{Cu-K}\alpha$ radiation. NaCl powder was used as a reference to calibrate the spacings. Intensities were estimated visually with a standard intensity scale. Lorentz and polarization corrections were carried out according to the method described by Arnott.⁵

All calculations were carried out on a FACOM 230-75 computer at Nagoya University.

RESULTS AND DISCUSSION

α Poly(L-valine)

Several diffraction rings were observed in an X-ray photograph of an unoriented film of high molecular weight poly(L-valine) (Figure 1). Most of the diffraction rings could be indexed on the basis of a two-

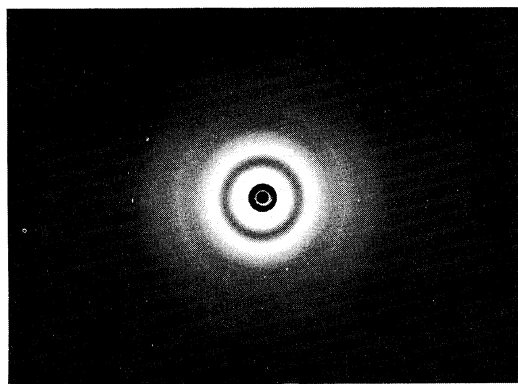


Figure 1. α Poly(L-valine): The X-ray diffraction photograph of unoriented specimen. (Cylindrical camera, $\text{Cu-K}\alpha$ radiation).

Table I. α Poly(L-valine): Observed and calculated spacings of the X-ray diffraction pattern^a

$d(\text{obsd})/\text{\AA}$	$d(\text{calcd})/\text{\AA}$	h	k	l	$I(\text{obsd})$
9.9	9.90	1	0	0	vs
4.93	5.72	1	1	0	s
	4.95	2	0	0	
	4.74 ^b	1	1	5 ^b	
3.4	3.39 ^b	1	1	5 ^b	m
	3.74	2	1	0	
3.2	3.30	3	0	0	vw
	2.86	2	2	0	
2.85	2.75	3	1	0	w
	2.27	3	2	0	
2.01	1.98	5	0	0	vw
1.52	1.50 ^b	0	0	18 ^b	vw

^a Hexagonal unit cell, $a=b=11.43\text{\AA}$, c =undeterminable.

^b These are indexed in the assumption that $c=27\text{\AA}$.

dimensional hexagonal lattice with $a=11.43\text{\AA}$ as is shown in Table I. This series of spacings is characteristic of the crystal in which cylindrical molecular chains are closely packed in a hexagonal fashion. If it is an $18_5(\alpha)$ -helix with repeat distance (c) of 27\AA , it may be reasonably assumed that two reflections, (105) $d=4.74\text{\AA}$ and (115) $d=3.39\text{\AA}$, which are usually fairly strong for the α -helical crystals, are included in the broad peaks around $d=4.93$ and 3.4\AA , respectively. Therefore, it is expected that the high-molecular-weight poly(L-valine) does not take an extended conformation like the β -sheet type, but rather an α -helix or similar helical structure.

Including the present polypeptide, the a -axis dimensions of the unit cells in α -polypeptides are roughly proportional to the sizes of the side chains, e.g., poly(L-alanine) $a=8.55\text{\AA}$,^{6,8} poly(L-phenylalanine) $a=11.55\text{\AA}$ (the present work), and poly(γ -methyl L-glutamate) $a=11.95\text{\AA}$.⁷

An irreversible and very distinct alteration in the diffraction pattern was recognized, when a film of α poly(L-valine) was soaked in 8% DCA in CCl_4 at room temperature, as shown in Figure 2. The new pattern which appeared was found to be that of the β -form poly(L-valine). Therefore, it is apparent that the conformation of high-molecular-weight poly(L-valine) is easily transformed from the α - to the β -

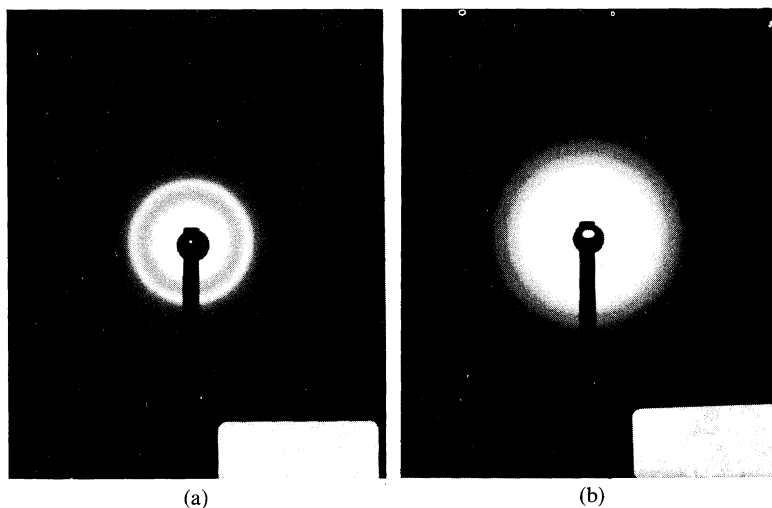


Figure 2. α Poly(L-valine): The alteration of X-ray diffraction pattern by soaking in 8% DCA in CCl_4 . (3.5 cm flat plate camera, Cu-K α radiation) (a) The original pattern, (b) the newly appeared pattern by the soaking.

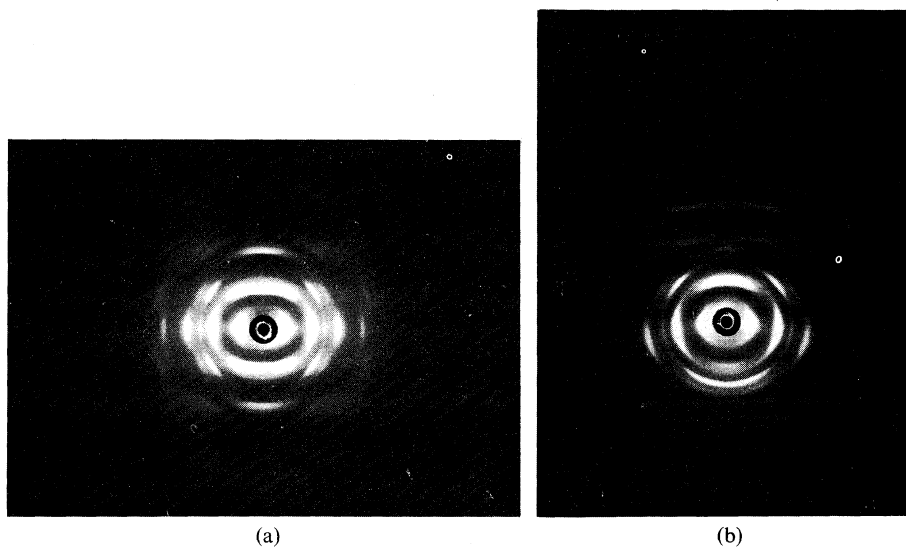


Figure 3. β Poly(L-valine): The X-ray diffraction photographs. (Cylindrical camera, Cu-K α radiation). (a) Fiber axis vertical, cylindrical film axis vertical, (b) fiber axis tilted 30° to the X-ray beam, cylindrical film axis horizontal.

form by treatment with a strong protonating solvent. Thus, poly(L-valine) can also take an α -form though it is appreciably unstable.

The present results support the proposal that the high-molecular-weight poly(L-valine) takes an α -helical structure, according to infrared and Raman

studies.³

β Poly(L-valine)

A normal rotation photograph of a fiber specimen of β poly(L-valine) and a photograph of the specimen tilted at 30° are shown in Figure 3. The latter

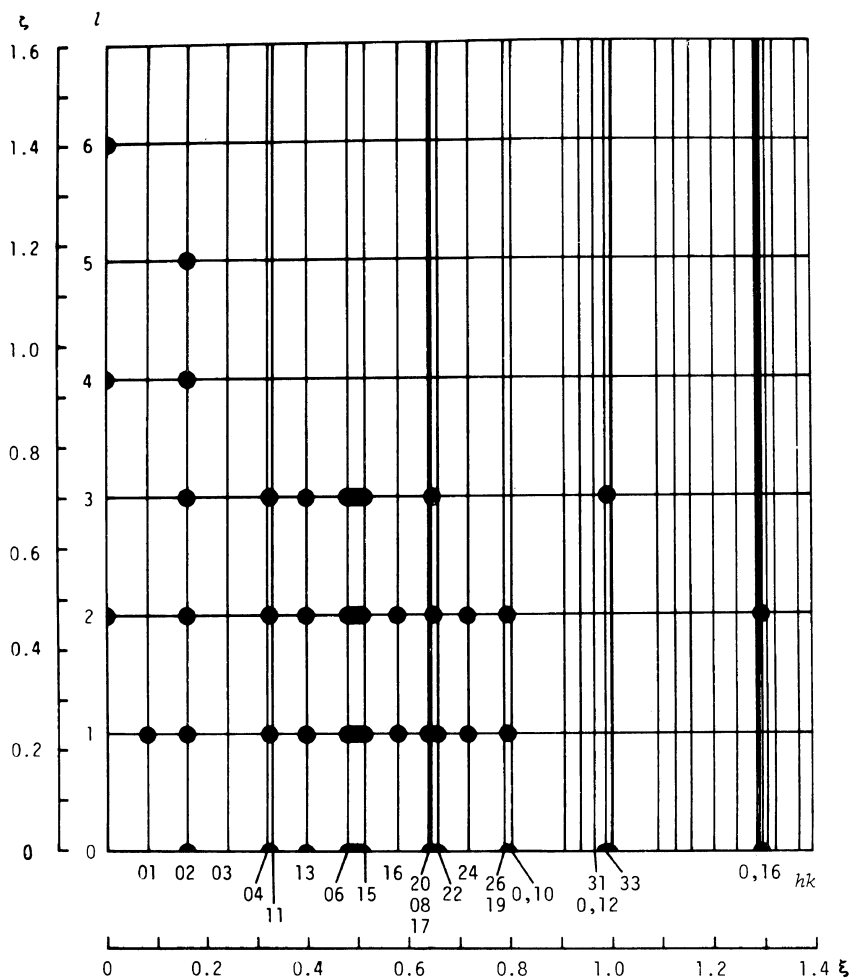


Figure 4. β Poly(L-valine): The reciprocal lattice rotation diagram. Orthorhombic unit cell. $a=4.80 \text{ \AA}$, $b=19.14 \text{ \AA}$, $c=6.59 \text{ \AA}$ (fiber axis). (ξ , ζ), reciprocal lattice unit.

photograph shows most of the near-meridional reflections, together with the meridional reflection at 1.10 \AA . The degree of orientation is appreciably high and the positions in the reciprocal lattice rotation diagram of most of the reflections were defined within narrow limits (Figure 4). Equatorial reflections were indexed on the basis of a two-chain centered orthorhombic unit cell having dimensions of $a=4.80 \text{ \AA}$ and $b=19.14 \text{ \AA}$, using β poly(L-alanine)⁸ as an analogy. In this unit cell, two reflections (040) and (110) at low diffraction angles lie in very close proximity, and the two are not resolved on the photograph. Furthermore, as is shown in Table II

and Figure 4, several accidental overlaps of reflections are observed at higher diffraction angles. The structure amplitudes of (020), (110), and (040) reflections which are large in β poly(L-alanine),¹ are also large in this specimen.

Most of the layer reflections appear on well-defined row lines associated with the equatorial reflections in the rotation photographs, but there are a few reflections on row lines for which the corresponding equatorial reflections are absent. The c -axis (fiber or chain axis) length was found to be 6.59 \AA from precise measurements of meridional reflections: (002), (004), and (006). Finally all the

Structures of Polypeptides

Table II. β Poly(L-valine): Spacings and intensities of the X-ray diffraction pattern

<i>l</i>	ξ	$d(\text{obsd})$	$I(\text{obsd})^a$	$d(\text{calcd})$	<i>h</i> <i>k</i> <i>l</i> ^b
	r.u.u. ^c	Å		Å	
0	0.16	9.6	1158	9.57	0 2 0
	0.33	4.7	699	4.79	0 4 0
				4.66	1 1 0
	0.41	3.81	26	3.84	1 3 0
	0.47~0.51	3.3~3.0	87	3.19	0 6 0
				2.99	1 5 0
	0.63~0.66	2.4~2.3	22	2.40	2 0 0
				2.40	0 8 0
				2.38	1 7 0
				2.33	2 2 0
				2.15	2 4 0
	0.70	2.2	1	2.03	2 5 0
	0.75	2.0	1	1.94	1 9 0
	0.80	1.93	2	1.92	2 6 0
				1.91	0 10 0
	0.97	1.59	9	1.60	0 12 0
				1.59	3 1 0
	1.0	1.53	1	1.55	3 3 0
				1.50	2 10 0
				1.48	3 5 0
1.3	1.2	1	1.20	4 0 0	
			1.20	0 16 0	
			1.19	4 2 0	
			1.19	2 14 0	
			1.18	3 11 0	
			1.16	4 4 0	
1	0.0	6.6	87	6.59	0 0 1
				6.23	0 1 1
	0.16	5.4	175	5.43	0 2 1
	0.33	3.8	87	3.87	0 4 1
				3.80	1 1 1
	0.40	3.3	18	3.32	1 3 1
	0.51	2.7	5	2.87	0 6 1
				2.73	1 5 1
	0.58	2.5	2	2.46	1 6 1
				2.26	2 0 1
	0.64~0.66	2.3~2.2	9	2.25	0 8 1
				2.24	1 7 1
				2.20	2 2 1
0.72	2.0	5	2.04	2 4 1	
			1.87	1 9 1	
0.8	1.8	2	1.84	2 6 1	
			1.84	0 10 1	
0.23	5.1	25	streak		
2	0.0	3.40	699	3.30	0 0 2
	0.16	3.1	36	3.12	0 2 2
	0.325	2.7	13	2.71	0 4 2
				2.69	1 1 2
0.40	2.5	6	2.50	1 3 2	

Table II. (continued)

<i>l</i>	ξ	<i>d</i> (obsd)	<i>I</i> (obsd) ^a	<i>d</i> (calcd)	<i>h k l</i> ^b
	r.u.u. ^c	Å		Å	
	0.51	2.2	5	2.29	0 6 2
				2.22	1 5 2
	0.57	2.1	2	2.07	1 6 2
				1.94	2 0 2
	0.65	1.9	2	1.94	0 8 2
				1.93	1 7 2
				1.90	2 2 2
	0.69	1.8	1	1.80	2 4 2
				1.68	1 9 2
	0.78	1.7	1	1.66	0 10 2
				1.66	2 6 2
				1.12	0 16 2
	1.3	1.1	1	1.12	4 2 2
				1.12	2 14 2
				1.11	3 11 2
				1.11	
3	0.16	2.1	2	2.14	0 2 3
				2.00	0 4 3
	0.33	2.0	6	1.99	1 1 3
				1.91	1 3 3
	0.40	1.9	5	1.81	0 6 3
				1.77	1 5 3
	0.50	1.8	5	1.62	2 0 3
				1.62	0 8 3
				1.61	1 7 3
				1.60	2 2 3
0.65	1.6	1	1.27	3 3 3	
			1.24	2 10 3	
			1.23	3 5 3	
1.0	1.3	1			
4	0.0	1.64	6	1.65	0 0 4
	0.16	1.6	1	1.62	0 2 4
5	0.16	1.3	1	1.31	0 2 5
6	0.0	1.10	6	1.10	0 0 6

^a Lp correction was not carried out. ^b Indices with $h+k=2n+1$ are excluded, except for (1 6 *l*), (0 1 1), and (2 5 0). ^c The r.l.u. is the abbreviation of the reciprocal lattice. unit.

reflections were indexed on the orthogonal unit cell:

$$a=4.80 \text{ \AA}, b=19.14 \text{ \AA}, c=6.59 \text{ \AA}.$$

The space group is $P2_12_12_1$ from the systematic absences, $00l$: $l=\text{odd}$, $0k0$: $k=\text{odd}$, $h00$: $h=\text{odd}$. The density measurement shows that the unit cell contains four L-valyl residues (Table III). The refinement of the lattice constants a and b was carried out making use of a diffractometer. The observed and calculated spacings of 40 spots of reflections together with intensities are tabulated in Table II.

The unit cell dimensions show clearly that poly(L-

Table III. β Poly(L-valine): Crystal data

Formula weight of residue	$M=99.13$ (C_5H_9ON) _{<i>n</i>}
Space group	$P2_12_12_1$
Unit cell dimensions	$a=4.80 \text{ \AA}$ $b=19.14 \text{ \AA}$ $c=6.59 \text{ \AA}$
Calculated density	$D_x=1.09 \text{ g cm}^{-3}$
Measured density	$D_m=1.18 \text{ g cm}^{-3}$
Number of residues in the unit cell	$Z=4$

valine) molecular chains make up a β pleated sheet structure in which the hydrogen-bonded sheet lies in an ac -plane of the unit cell. The interchain distance in the hydrogen-bonded sheet corresponds to the a -axis length, 4.80 Å, and the inter-sheet distance corresponds to half the length of b -axis 9.57 Å. According to Pauling's β pleated sheet models,⁹ the c -axis length may be that of the parallel pleated-sheet structure. However, it can not be determined at the present stage as to which arrangement occurs in the pleated sheet, parallel or antiparallel.

The model structure of α poly(L-valine) is so constructed as to satisfy the following conditions,

(a) The molecular chain coincides with a crystallographic two-fold screw axis.

(b) The period of the c -axis corresponds to the length of two consecutive valyl residues along the fiber axis.

Therefore, the conformational parameters (ϕ , ψ) of the main chain were evaluated as $\phi = -116.8^\circ$ and $\psi = 114.4^\circ$, using the equations for helical parameters as functions of internal coordinates¹⁰ where the following conditions are retained: $\omega = 180^\circ$, the number of repeating units per turn: $n = 2$ (2_1 -helix), and the rise along the helix axis per repeating unit: $d = 3.3$ Å ($c/2$, the length of one valyl residue along the c -axis). The torsional angle N-C $^\alpha$ -C $^\beta$ -C $^\gamma_1$ (χ_{11}) was evaluated as -170° by the potential energy calculation. The other structural parameters of valyl residue used in the model construction are tabulated in Table IV.

Two crystal structure models are considered acceptable:

A: the model in which the parallel-chain sheets are packed antiparallel,

Table IV. Dimensions of L-valyl residue used in the model construction

Bond distance/Å		Bond angle/ $^\circ$				
N — C $^\alpha$	1.49	C $^\alpha$ — N	— C'	— C'	121.6	
N — C'	1.33	N — C'	— O	— O	122.6	
C' — O	1.22	N — C'	— C $^\alpha$	— C $^\alpha$	117.7	
C' — C $^\alpha$	1.53	O — C'	— C $^\alpha$	— C $^\alpha$	119.6	
C $^\alpha$ — C $^\beta$	1.56	C' — C $^\alpha$	— N	— N	109.8	
C $^\beta$ — C $^\gamma_1$	1.52	N — C $^\alpha$	— C $^\beta$	— C $^\beta$	109.7	
C $^\beta$ — C $^\gamma_2$	1.52	C' — C $^\alpha$	— C $^\beta$	— C $^\beta$	114.6	
		C $^\alpha$ — C $^\beta$	— C $^\gamma_1$	— C $^\gamma_1$	110.7	
		C $^\alpha$ — C $^\beta$	— C $^\gamma_2$	— C $^\gamma_2$	110.7	
		C $^\gamma_1$ — C $^\beta$	— C $^\gamma_2$	— C $^\gamma_2$	113.1	

B: Arnott's disordered model.¹

The feature of the pseudo-centered lattice symmetry in the ($hk0$) reflections prefers the model B going on the analogy of the structure of β poly(L-alanine),¹ in which each chain site contains half an up-pointing chain and half a down-pointing chain. Using several $hk0$ reflections, the crystal structure was refined by the R-map method. The refined parameters are the rotation angle of the main chain of poly(L-valine) around the two-fold screw axis θ and the torsion angle χ_{11} . The angle θ is defined as an angle between (010) plane and the normal vector from the chain axis to C $^\alpha$ -carbon atom. For the calculation of the discrepancy factor R , accidental overlaps of reflections were taken into account by

$$R = \frac{\sum_j \left| \sqrt{I_j^0} - \sqrt{\sum_i m_{ij} |F_{ij}^c|^2} \right|}{\sum_j \sqrt{I_j^0}}$$

where I_j^0 is the Lp corrected intensity of the j -th spot of overlap reflection, and m_{ij} and $|F_{ij}^c|$ are the multiplicity factor and the calculated structure amplitude for the i -th reflection in the j -th spot, respectively.

A structure with a minimum R -value 0.31 was found to be stereochemically reasonable. The final parameters θ and χ_{11} were 82.8° and -170.0° , respectively. The atomic parameters at the present stage are tabulated in Table V. Table VI gives the values $\sqrt{I_j^0}$, and $\sqrt{\sum_i m_{ij} |F_{ij}^c|^2}$.

The c -axis projection of the structure is superimposed on the Fourier synthesis $\rho(xy)$ in Figure 5, which shows clearly (though with low resolution) the centered core of the main chain and the stacks of isopropyl side chains. In this Fourier synthesis, the observed structure amplitudes $|F_{ij}^o|$'s which can not be resolved, were evaluated by the formula

$$|F_{ij}^o| = \sqrt{I_j^0} \frac{|F_{ij}^c|}{\sqrt{\sum_i m_{ij} |F_{ij}^c|^2}}$$

A large contraction of the c -axis occurs in β poly(L-valine) as compared with that of β poly(L-alanine) ($c = 6.89 \pm 0.02$ Å).¹ The peptide plane in β poly(L-valine) forms an angle of 30° with the fiber axis, while that of β poly(L-alanine) is 20° .

Figure 6 shows the antiparallel pleated sheet of poly(L-valine). Circles represent the spheres of van der Waals' radius 2.0 Å of methyl group. As is

Table V. β Poly(L-valine): Atomic coordinates in the unit cell
U1 residue on the axis ($a/4, 0, z$)

Atom	$x_0/\text{\AA}$	$y_0/\text{\AA}$	$z_0/\text{\AA}$
N	1.63	-0.21	-1.23
C $^\alpha$	1.32	-0.99	0
C $^\beta$	1.97	-2.40	-0.10
C $^\gamma$ 1	1.51	-3.355	0.99
C $^\gamma$ 2	1.73	-3.01	-1.48
C'	1.74	-0.19	1.24
O	2.92	0.01	1.46
Residues on the axis ($a/4, 0, z$)			
U1: ^a	$x_0,$	$y_0,$	z_0
U2:	$-x_0+a/2,$	$-y_0,$	$z_0+c/2$
D1: ^a	$x_0,$	$-y_0,$	$-z_0+c/2+\Delta z^b$
D2:	$-x_0+a/2,$	$y_0,$	$-z_0+\Delta z$
Residues on the axis ($3a/4, b/2, z$)			
U3:	$x_0+a/2,$	$y_0+b/2,$	$z_0-c/2-\Delta z$
U4:	$-x_0+a,$	$-y_0+b/2,$	$z_0-\Delta z$
D3:	$x_0+a/2,$	$-y_0+b/2,$	$-z_0$
D4:	$-x_0+a,$	$y_0+b/2,$	$-z_0+c/2$

^a U and D represent up-pointing and down-pointing chains, respectively.

^b The small value Δz adjusts the hydrogen-bonding condition. C $^\alpha$ is tentatively located at $z=0$.

shown, the side chains of L-valyl residues have close contacts with the adjacent side chains in the same sheet. The contraction of the main chain may be attributable to the formation of stable van der Waals' contacts among the bulky hydrophobic side chains. On the other hand, in β poly(L-alanine), no such large contraction occurs, since the size of a methyl side chain is too small to induce close contacts among these side chains. The sheet structure of poly(L-alanine) may be stabilized by the interlocking of the side chains with those in the opposite sheet and this may be called inter-sheet stacking.

When a homopolypeptide with a large hydrophobic side group forms the β pleated sheets, one of the most stable accommodations of the bulky side groups is achieved by the intra-sheet stacking of the side chains, which brings about the contraction of the main chain, in this case.

β Poly(L-isoleucine)

An X-ray diffraction photograph of a fiber specimen of β poly(L-isoleucine) is shown in Figure 7. The

Table VI. β Poly(L-valine): Comparison between $\sqrt{I^0}$ and $\sqrt{\sum_i m_i |F_i^c|^2}$ for $hk0$ reflections^a

h	k	$\sqrt{I^0}$	$\sqrt{\sum_i m_i F_i^c ^2}$
0	2	196	150
0	4		174
1	1	219	
1	3	47	30
0	6	100	156
1	5		
2	0		
0	8	58	81
1	7		
2	2		
2	4	20	19
1	9		
2	6	22	44
0	10		
2	8		
1	11		
0	12	46	54
3	1		
3	3		

^a The isotropic temperature factor was assumed to $B=6.0 \text{\AA}^2$.

orientation of the fiber axis is rather poor. The equatorial reflections were indexed on an orthogonal cell with the dimensions of $a=4.8 \text{\AA}$ and $b=23 \text{\AA}$. The c -axis repeat distance was determined to be 6.6\AA from the layer line spacings. However, the space group could not be established owing to the low resolution of the diffraction pattern. The b -axis is longer than that of β poly(L-valine), reflecting the sizes of the side chains, but there is no significant difference in the a - and b -axes. Thus, poly(L-isoleucine) makes up a β pleated sheet structure similar to that of β poly(L-valine). Observed and calculated spacings are tabulated in Table VII.

In order to obtain a highly oriented fiber specimen, 8% DCA in CCl_4 was used as the swelling agent. The stretching ratio, however, resulted in being too much less than that of β poly(L-valine), since the degree of swelling is somewhat low. The difficulty in swelling may be explained as follows. It is supposed that

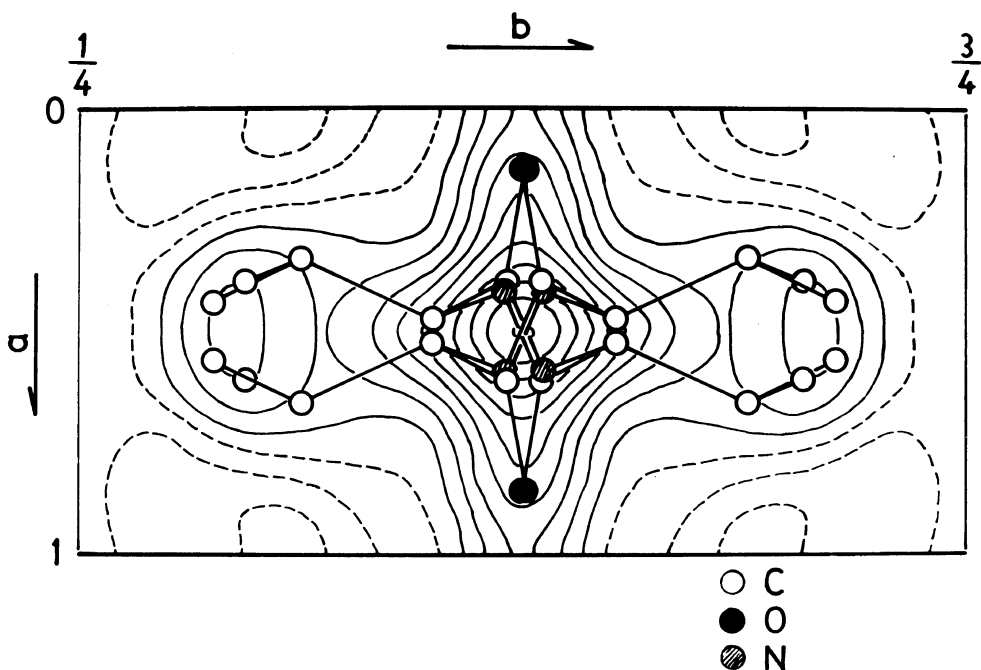


Figure 5. β Poly(L-valine): The c -axis projection of the structure superimposed on the Fourier synthesis $\rho(xy)$. $F(000)$ is not included. Positive and negative contours are shown as solid and broken lines, respectively. Contours are at arbitrary but equal intervals.

extended hydrophobic barriers are constructed in both sides of any β pleated sheets, which have large hydrophobic side chains such as poly(L-valine) and poly(L-isoleucine), by virtue of intra-sheet van der Waals' forces among the bulky side chains. These forces in β poly(L-isoleucine) increase more than those of β poly(L-valine) when the isopropyl side chain is converted into the more bulky isobutyl group. Thus, it becomes more difficult to destroy the barriers and to solvate the hydrogen-bonding moieties of the backbone.

A film specimen of poly(L-isoleucine) was also obtained by the same method by which the α -form specimen of poly(L-valine) was prepared. The diffraction pattern of this specimen is shown in Figure 8, and is quite different from that of the β -form. The indexing on the basis of a two-dimensional hexagonal lattice has not yet been accomplished. The structural make up of this diffraction pattern is under investigation.

Poly(L-phenylalanine)

An X-ray diffraction photograph of a fiber speci-

men of poly(L-phenylalanine) is shown in Figure 9. The innermost spot on the equatorial line was found to split into two reflections, using a flat plate camera with a long distance from specimen to film and a fine collimator. When the fiber axis was tilted at about 30° to the X-ray beam the photograph showed a strong reflection on the meridian with a spacing of 1.5 \AA . This reflection corresponds to the residue translation in the α -helix. Then, the indexing of the reflections were carried out in terms of the α -helical structure. But several reflections including the innermost one mentioned above could not be indexed. This is easily explained by assuming the coexistence of the α - and ω -helix forms, and all the features found in the diffraction pattern are explained as follows. The twin reflections on the equator are interpreted as (100) of the ω -form (12.86 \AA), and (100) of the α -form (10.00 \AA), respectively. A strong layer reflection with a spacing of 5.13 \AA is one of the first layer reflections of the ω -form. The observed reflections are tabulated in Table VIII. Reflections from the ω -form are indexed on the basis of tetragonal lattice with dimensions of $a=b=12.86 \text{ \AA}$ and

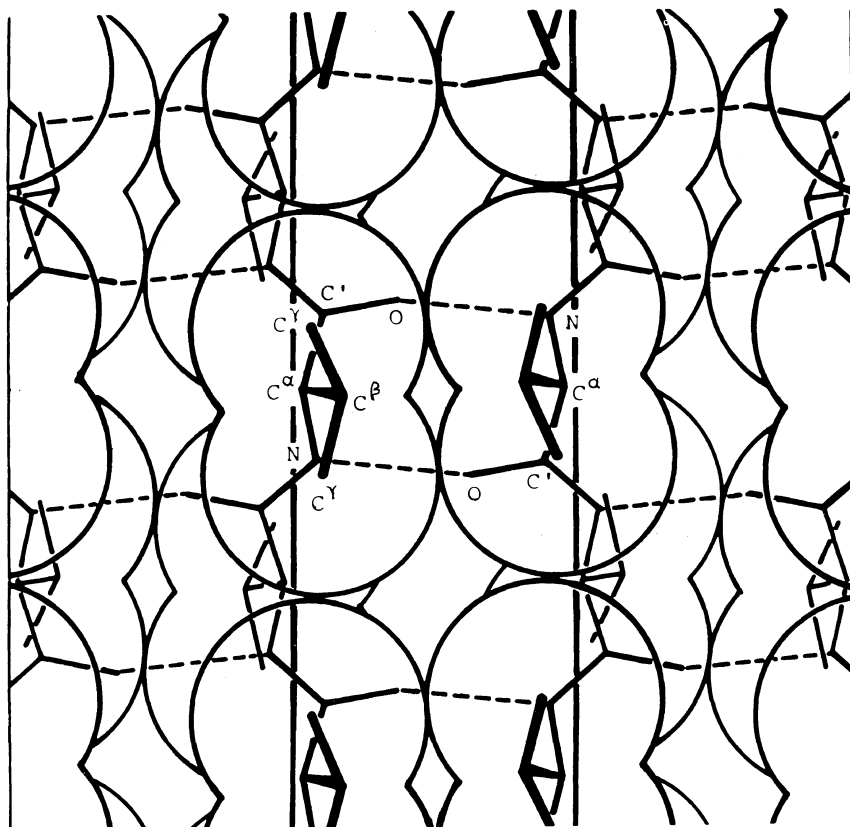


Figure 6. Structure model of β pleated sheet of poly(L-valine) viewed normal to the sheet. Circles represent the spheres of van der Waals' radius 2.0 \AA of methyl groups. Molecular chains are vertical and the direction of hydrogen bonding is horizontal.



Figure 7. β Poly(L-isoleucine): The X-ray diffraction photograph. (Cylindrical camera, Cu-K α radiation) Fiber axis, vertical).

Table VII. β Poly(L-isoleucine): Observed and calculated spacings of the X-ray diffraction pattern^a

$d(\text{obsd})/\text{\AA}$	$d(\text{calcd})/\text{\AA}$	h	k	l	$I(\text{obsd})$
11.6	11.4	0	2	0	vs
5.70	5.75	0	4	0	w
4.75	4.70	1	1	0	vs
3.75	3.83	0	6	0	s
3.30	3.32	1	5	0	w
2.82	2.87	0	8	0	vw
2.44	2.40	2	0	0	vw

^a Orthorhombic unit cell. $a=4.8 \text{ \AA}$, $b=23 \text{ \AA}$, $c=6.6 \text{ \AA}$.

$c=5.59 \text{ \AA}$ (fiber axis), and for the α -form, the two-dimensional hexagonal lattice with $a=b=11.55 \text{ \AA}$ is applied. A few weak layer lines were also observed between the equatorial line and the first layer line of

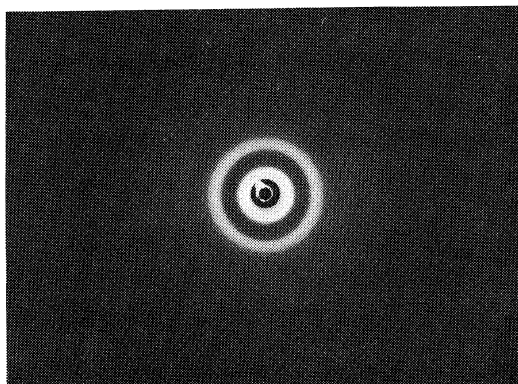


Figure 8. Poly(L-isoleucine): The X-ray diffraction photograph of the specimen of an unidentified form. (Cylindrical camera, Cu-K α radiation).

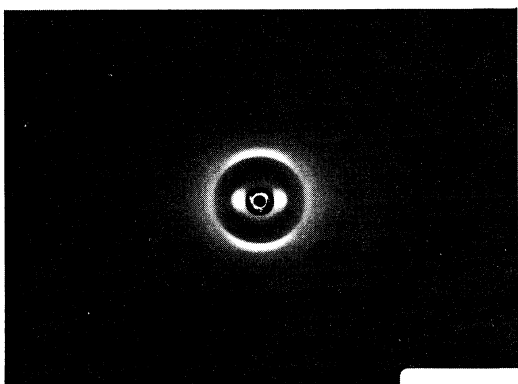


Figure 9. Poly(L-phenylalanine): The X-ray diffraction photograph. (Cylindrical camera, Cu-K α radiation) Fiber axis, vertical).

the ω -form, which seemed to correspond to those from the α -form. However, because of the somewhat poor resolution of the layer lines, the c -axis repeat distance could not be determined at this stage. This specimen contains both ω - and α -helices, but the

Table VIII. Poly(L-phenylalanine): Observed and calculated spacings of the X-ray diffraction pattern (ω - and α -helix forms)^a

$d(\text{obsd})/\text{\AA}$	$d(\text{calcd})/\text{\AA}$	Helix	h	k	l	$I(\text{obsd})$
Equatorial reflections						
12.86	12.86	ω	1	0	0	vs
10.00	10.00	α	1	0	0	s
6.43	6.43	ω	2	0	0	w
4.93	5.00	α	2	0	0	w
Near meridional reflections						
5.13	5.13	ω	1	0	1	vs
4.48		α				w
3.88		α				w
3.55		α				w
2.72	2.73	ω	1	0	2	w
1.50		α				m

^a Unit cell dimensions:

ω -Helix; tetragonal, $a = 12.86 \text{ \AA}$, $c = 5.59 \text{ \AA}$. (calculated density $D_x = 1.06 \text{ g cm}^{-3}$, $Z = 4$)

α -Helix; hexagonal, $a = 11.55 \text{ \AA}$, $c = \text{undeterminable}$.

crystalline regions of the latter are not well developed as compared with those of the former. Besides the X-ray diffraction study, infrared and Raman spectroscopic studies also suggested the coexistence of two helix forms (one of them is the α -helix) in a specimen of high molecular weight poly(L-phenylalanine).¹⁴ In addition, these spectroscopic studies report that the proportion of the two helix forms reverses with aging of this specimen.

The ω -helical conformation is very rarely to be found in the structure of polypeptides, and this specimen may be the first example of homopolymers of unmodified α -amino acids. Two poly(α -amino acid)s, poly(β -benzyl L-aspartate) and poly(*S*-benzylthio-L-cysteine) are known to have ω -helical conformations. The cell dimensions of these ω -helical structures are tabulated in Table IX.

Table IX. Cell dimensions (tetragonal) of ω -helical structures

Polymer ^a	Cell dimensions		Reference
	$a/\text{\AA}$	$c/\text{\AA}$	
Poly(β -benzyl L-aspartate)	13.85	5.30	11, 12
Poly(<i>S</i> -benzylthio-L-cysteine)	14.28	5.55	13
Poly(L-phenylalanine)	12.86	5.59	this study

^a The side chain structures: β -benzyl-Asp, $-\text{C}^{\beta}\text{H}_2-\text{CO}-\text{O}-\text{CH}_2-\text{C}_6\text{H}_5$; *S*-benzylthio-Cys, $-\text{C}^{\beta}\text{H}_2-\text{S}-\text{S}-\text{CH}_2-\text{C}_6\text{H}_5$; Phe, $-\text{C}^{\beta}\text{H}_2-\text{C}_6\text{H}_5$.

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