

X-Ray Study of the Substitution Effect in Poly[γ -(*o*-chlorobenzyl) L-glutamate], Poly[γ -(*m*-chlorobenzyl) L-glutamate], and Poly[γ -(*p*-chlorobenzyl) L-glutamate] in the Solid State

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ABSTRACT: X-Ray diffraction measurements were made on fibers of poly[γ -(*o*-chlorobenzyl) L-glutamate], poly[γ -(*m*-chlorobenzyl) L-glutamate], and poly[γ -(*p*-chlorobenzyl) L-glutamate]. All reflections of the poly[γ -(*o*-chlorobenzyl) L-glutamate] fibers were indexed on an orthorhombic lattice of $a=26.50$ Å, $b=30.60$ Å, and $c=27.02$ Å. The observed density was 1.367 g cm⁻³ as compared with the calculated value of 1.382 g cm⁻³. All reflections of the poly[γ -(*m*-chlorobenzyl) L-glutamate] fibers were indexed on a hexagonal lattice of $a=b=15.50$ Å and $c=27.10$ Å. The observed density of 1.356 g cm⁻³ was larger than the calculated value of 1.343 g cm⁻³. All reflections of the poly[γ -(*p*-chlorobenzyl) L-glutamate] fibers were indexed on an orthorhombic lattice of $a=15.24$ Å, $b=26.40$ Å, and $c=10.67$ Å. The observed density of 1.362 g cm⁻³ was comparable with the calculated value of 1.371 g cm⁻³. By applying the methods of the cylindrical Patterson map and equatorial Fourier map, the position of the chlorine atom at the end of the side chain was estimated. The results obtained indicated that the radial distance of the chlorine atom from the helix axis is about 4 Å, 6 Å, and 10 Å for poly[γ -(*o*-chlorobenzyl) L-glutamate], poly[γ -(*m*-chlorobenzyl) L-glutamate], and poly[γ -(*p*-chlorobenzyl) L-glutamate], respectively. These results are interpreted in terms of side chain conformation.

KEY WORDS Poly[γ -(*o*-chlorobenzyl) L-glutamate] / Poly[γ -(*m*-chlorobenzyl) L-glutamate] / Poly[γ -(*p*-chlorobenzyl) L-glutamate] / X-Ray Diffraction / Cylindrical Patterson Map / Equatorial Fourier Map /

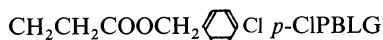
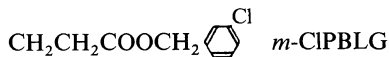
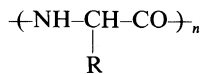
It has been reported that poly(β -benzyl L-aspartate) (PBLA) is a left-handed α -helix in the solid film cast from chloroform solution.¹ If the α -helix of PBLA is heated to a temperature of about 140°C, the conformation changes into the ω -helix in which four residues make one turn during a repeating period. On the other hand, poly[β -(*p*-chlorobenzyl) L-aspartate] (*p*-CIPBLA), in which the hydrogen atom at the *p*-position of the phenyl ring of PBLA is replaced by a chlorine atom is reported to be a right-handed α -helix.² *p*-CIPBLA does not change into the ω -helix when heated to 160°C for two hours, but does so when heated up to 190°C. Therefore, it is of interest to study how the chlorine atom at the phenyl ring of poly(γ -benzyl L-glutamate) (PBLG) affects the polymer conformation.

In previous papers, we reported the preliminary

results of X-ray diffraction studies on the fibers of poly[γ -(*o*-chlorobenzyl) L-glutamate] (*o*-CIPBLG), poly[γ -(*m*-chlorobenzyl) L-glutamate] (*m*-CIPBLG),³ and poly[γ -(*p*-chlorobenzyl) D-glutamate] (*p*-CIPBDG).⁴ We found that *o*-CIPBLG and *m*-CIPBLG are 18-residue 5-turn (18/5) α -helices and that *p*-CIPBDG is a 7-residue 2-turn (7/2) α -helix, instead of the conventional 18/5 α -helix. Thus, substitution of the hydrogen atom of the phenyl ring of PBLG was found to influence the main chain conformation.

The precise molecular structure of a polypeptide with a long and flexible side chain, such as PBLA and PBLG has yet to be found. An attempt was made to determine the molecular structure of *p*-CIPBLG, but without success. The present paper is a study in more detail of the substitution effect on

some polypeptides using the X-ray diffraction method.



EXPERIMENTAL

The preparation of *o*-CIPBLG, *m*-CIPBLG, and *p*-CIPBLG and their oriented fibers, along with the procedures of X-ray diffraction experiments, have been described in previous papers.^{3,4} Intensities were estimated by visual comparison on a standard intensity scale, and corrected for Lorentz and polarization effects. The densities of samples were determined by the floatation method with aqueous potassium acetate at 25°C.

RESULTS AND DISCUSSION

Unit Cell

Diffraction photographs of *o*-CIPBLG, *m*-CIPBLG, and *p*-CIPBDG have been reported in the previous papers.^{3,4} The diffraction photographs of *p*-CIPBLG fibers were very similar to those of D-isomer.

o-CIPBLG

106 sharp reflections were observed on the X-ray diffraction pattern of *o*-CIPBLG fibers. Although the equatorial reflections can be indexed on a hexagonal net of $a=b=15.30\text{ \AA}$, the row-line reciprocal coordinates of many other layer-line reflections do not always coincide with the reciprocal coordinates of the equatorial reflections, as shown in Figure 2 and in Table I. Therefore, the true unit cell is not hexagonal but orthorhombic. All the reflections can be indexed on an orthorhombic unit cell with $a=26.50\text{ \AA}$ and $b=30.60\text{ \AA}$ in which four molecules are contained. The hexagonal unit cell is a subunit cell of the orthorhombic unit cell. This unit cell is shown in Figure 1a.

The repeat distance along the fiber axis (*c*-axis) was 27.02 Å. The reciprocal lattice rotation diagram of *o*-CIPBLG is shown in Figure 2. Table I gives

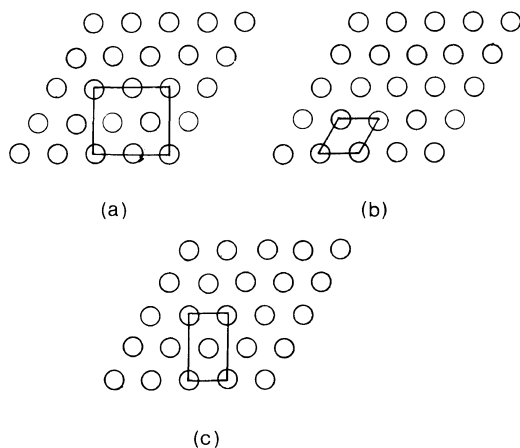


Figure 1. The unit cells of *o*-CIPBLG, *m*-CIPBLG, and *p*-CIPBLG: (a) an orthorhombic unit cell of *o*-CIPBLG crystal containing four molecules; (b) a hexagonal unit cell of *m*-CIPBLG crystal containing one molecule; (c) an orthorhombic unit cell of *p*-CIPBLG crystal containing two molecules.

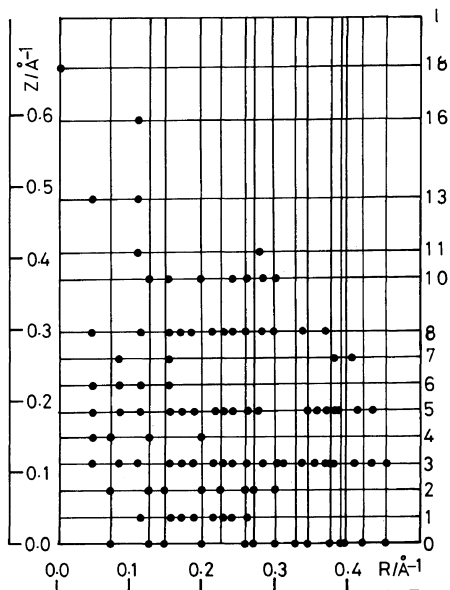


Figure 2. Reciprocal lattice rotation diagram of *o*-CIPBLG.

observed reciprocal coordinates R_o , calculated reciprocal coordinates R_c , observed intensities I_o , and indices.

The observed density of *o*-CIPBLG was 1.367 g cm^{-3} as compared with the calculated value of

X-Ray Study of Poly[(γ -chlorobenzyl) L-glutamate]s

Table I. X-Ray reflections of poly[γ -(*o*-chlorobenzyl) L-glutamate] fibers^a

| R_o^b | R_c^c | I_o^d | h | k | l | R_o | R_c | I_o | h | k | l |
|---------|---------|---------|-----|-----|-----|--------|--------|-------|-----|-----|-----|
| 0.0752 | 0.0755 | 597.1 | 2 | 0 | 0 | 0.0750 | 0.0755 | 0.7 | 2 | 0 | 4 |
| 0.1305 | 0.1307 | 779.8 | 3 | 2 | 0 | 0.1329 | 0.1307 | 0.6 | 3 | 2 | 4 |
| 0.1506 | 0.1509 | 932.6 | 4 | 0 | 0 | 0.1991 | 0.1997 | 2.6 | 5 | 2 | 4 |
| 0.1994 | 0.1997 | 118.5 | 5 | 2 | 0 | 0.0465 | 0.0499 | 32.3 | 1 | 1 | 5 |
| — | 0.2264 | 0.0 | 6 | 0 | 0 | 0.0851 | 0.0822 | 17.9 | 2 | 1 | 5 |
| 0.2614 | 0.2614 | 202.0 | 6 | 4 | 0 | 0.1174 | 0.1178 | 60.5 | 3 | 1 | 5 |
| 0.2722 | 0.2721 | 766.5 | 7 | 2 | 0 | 0.1561 | 0.1544 | 148.3 | 4 | 1 | 5 |
| 0.3019 | 0.3019 | 195.9 | 8 | 0 | 0 | 0.1735 | 0.1729 | 129.4 | 3 | 4 | 5 |
| 0.3287 | 0.3290 | 121.7 | 8 | 4 | 0 | 0.1896 | 0.1887 | 141.1 | 5 | 0 | 5 |
| 0.3454 | 0.3459 | 89.2 | 9 | 2 | 0 | 0.2186 | 0.2224 | 57.3 | 4 | 5 | 5 |
| 0.3779 | 0.3774 | 13.5 | 10 | 0 | 0 | 0.2295 | 0.2295 | 106.9 | 5 | 4 | 5 |
| 0.3914 | 0.3922 | 10.0 | 9 | 6 | 0 | 0.2473 | 0.2467 | 14.4 | 6 | 3 | 5 |
| 0.3992 | 0.3994 | 20.9 | 10 | 4 | 0 | 0.2648 | 0.2641 | 5.8 | 7 | 0 | 5 |
| 0.4204 | 0.4202 | 12.3 | 11 | 2 | 0 | 0.2792 | 0.2792 | 1.1 | 6 | 5 | 5 |
| 0.4526 | 0.4528 | 12.9 | 12 | 0 | 0 | 0.3461 | 0.3459 | 0.8 | 6 | 8 | 5 |
| 0.1174 | 0.1178 | 0.7 | 3 | 1 | 1 | 0.3578 | 0.3600 | 2.0 | 8 | 6 | 5 |
| 0.1580 | 0.1544 | 2.6 | 4 | 1 | 1 | 0.3716 | 0.3717 | 5.7 | 7 | 8 | 5 |
| 0.1737 | 0.1729 | 1.5 | 3 | 4 | 1 | 0.3819 | 0.3830 | 5.7 | 10 | 2 | 5 |
| 0.1888 | 0.1887 | 1.7 | 5 | 0 | 1 | 0.3895 | 0.3899 | 5.8 | 10 | 3 | 5 |
| 0.2196 | 0.2224 | 2.2 | 4 | 5 | 1 | 0.4157 | 0.4151 | 4.9 | 11 | 0 | 5 |
| 0.2308 | 0.2295 | 2.6 | 5 | 4 | 1 | 0.4333 | 0.4352 | 2.3 | 5 | 12 | 5 |
| 0.2465 | 0.2467 | 2.9 | 6 | 3 | 1 | 0.0497 | 0.0499 | 9.3 | 1 | 1 | 6 |
| 0.2646 | 0.2642 | 3.4 | 7 | 0 | 1 | 0.0841 | 0.0822 | 2.3 | 2 | 1 | 6 |
| 0.0766 | 0.0755 | 1.0 | 2 | 0 | 2 | 0.1171 | 0.1178 | 10.7 | 3 | 1 | 6 |
| 0.1291 | 0.1307 | 6.4 | 3 | 2 | 2 | 0.1548 | 0.1544 | 5.4 | 4 | 1 | 6 |
| 0.1510 | 0.1509 | 12.3 | 4 | 0 | 2 | 0.0842 | 0.0822 | 11.7 | 2 | 1 | 7 |
| 0.2002 | 0.1997 | 108.7 | 5 | 2 | 2 | 0.1569 | 0.1544 | 1.4 | 4 | 1 | 7 |
| 0.2257 | 0.2264 | 52.3 | 6 | 0 | 2 | 0.3832 | 0.3830 | 13.5 | 10 | 2 | 7 |
| 0.2605 | 0.2614 | 22.1 | 6 | 4 | 2 | 0.4056 | 0.4060 | 11.3 | 5 | 11 | 7 |
| 0.2718 | 0.2721 | 26.4 | 7 | 2 | 2 | 0.0487 | 0.0499 | 3.8 | 1 | 1 | 8 |
| 0.3019 | 0.3019 | 11.5 | 8 | 0 | 2 | 0.1181 | 0.1178 | 17.6 | 3 | 1 | 8 |
| 0.0462 | 0.0499 | 1.1 | 1 | 1 | 3 | 0.1555 | 0.1544 | 23.4 | 4 | 1 | 8 |
| 0.0852 | 0.0822 | 9.8 | 2 | 1 | 3 | 0.1734 | 0.1729 | 6.8 | 3 | 4 | 8 |
| 0.1124 | 0.1132 | 2.8 | 3 | 0 | 3 | 0.1919 | 0.1915 | 27.0 | 5 | 1 | 8 |
| 0.1572 | 0.1544 | 109.5 | 4 | 1 | 3 | 0.2212 | 0.2224 | 6.0 | 4 | 5 | 8 |
| 0.1732 | 0.1729 | 55.9 | 3 | 4 | 3 | 0.2306 | 0.2295 | 7.3 | 5 | 4 | 8 |
| 0.1887 | 0.1887 | 14.1 | 5 | 0 | 3 | 0.2453 | 0.2467 | 13.2 | 6 | 3 | 8 |
| 0.2203 | 0.2224 | 5.2 | 4 | 5 | 3 | 0.2646 | 0.2641 | 9.7 | 7 | 0 | 8 |
| 0.2295 | 0.2295 | 14.5 | 5 | 4 | 3 | 0.2853 | 0.2849 | 6.7 | 3 | 8 | 8 |
| 0.2443 | 0.2467 | 2.3 | 6 | 3 | 3 | 0.3019 | 0.3019 | 5.1 | 4 | 8 | 8 |
| 0.2648 | 0.2641 | 10.3 | 7 | 0 | 3 | 0.3398 | 0.3396 | 0.9 | 9 | 0 | 8 |
| 0.2857 | 0.2849 | 5.0 | 3 | 8 | 3 | 0.3693 | 0.3717 | 0.7 | 7 | 8 | 8 |
| 0.3034 | 0.3036 | 14.9 | 8 | 1 | 3 | 0.1309 | 0.1307 | 9.3 | 3 | 2 | 10 |
| 0.3142 | 0.3152 | 11.1 | 3 | 9 | 3 | 0.1554 | 0.1544 | 8.7 | 4 | 1 | 10 |
| 0.3399 | 0.3396 | 6.8 | 9 | 0 | 3 | 0.1953 | 0.1961 | 6.7 | 0 | 6 | 10 |
| 0.3558 | 0.3535 | 11.1 | 9 | 3 | 3 | 0.2466 | 0.2467 | 3.3 | 6 | 3 | 10 |
| 0.3729 | 0.3717 | 6.2 | 7 | 8 | 3 | 0.2646 | 0.2641 | 4.5 | 7 | 0 | 10 |
| 0.3788 | 0.3789 | 5.4 | 8 | 7 | 3 | 0.2863 | 0.2849 | 6.6 | 3 | 8 | 10 |
| 0.3857 | 0.3830 | 14.9 | 10 | 2 | 3 | 0.3068 | 0.3036 | 5.3 | 8 | 1 | 10 |
| 0.4137 | 0.4151 | 9.9 | 11 | 0 | 3 | 0.1113 | 0.1132 | 0.9 | 3 | 0 | 11 |
| 0.4349 | 0.4352 | 4.0 | 11 | 4 | 3 | 0.2795 | 0.2792 | 2.2 | 6 | 5 | 11 |
| 0.4563 | 0.4575 | 3.6 | 13 | 2 | 3 | 0.0450 | 0.0499 | — | 1 | 1 | 13 |
| 0.0478 | 0.0499 | 2.7 | 1 | 1 | 4 | 0.1169 | 0.1178 | — | 3 | 1 | 13 |

Table I. (Continued)

| R_o^b | R_c^c | I_o^d | h | k | l | R_o^b | R_c^c | I_o^d | h | k | l |
|---------|---------|---------|-----|-----|-----|---------|---------|---------|-----|-----|-----|
| 0.0000 | 0.0000 | — | 0 | 0 | 18 | 0.1073 | 0.1051 | — | 1 | 3 | 16 |

^a Unit cell dimensions: $a=26.50 \text{ \AA}$, $b=30.60 \text{ \AA}$, and $c=27.02 \text{ \AA}$.

^b Observed reciprocal coordinates.

^c Calculated reciprocal coordinates.

^d Observed intensities.

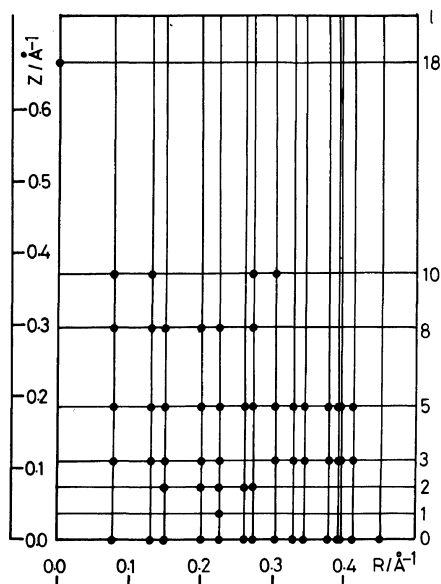


Figure 3. Reciprocal lattice rotation diagram of *m*-CIPBLG.

1.382 g cm^{-3} .

In highly oriented enantiomeric α -helical polypeptides, forbidden meridional reflections (with a spacing of about 4.4 \AA or 2.2 \AA) have been observed,^{2,5-7} and interpreted in terms of departure from ideal helical symmetry. However, in the diffraction pattern of *o*-CIPBLG fibers, these reflections were observed not on but near the meridian, suggesting that both the main and side chains should be within the ideal $18/5$ helical symmetry.

m-CIPBLG

58 reflections were observed on the X-ray diffraction pattern of the *m*-CIPBLG fibers. The equatorial and other layer line reflections were indexed on a hexagonal unit cell of $a=b=15.50 \text{ \AA}$, as shown in Figure 1b. Some layer-lines have sharp reflections

and diffuse streaks.

The repeat distance along the fiber axis (*c*-axis) was 27.10 \AA . The reciprocal lattice rotation diagram of *m*-CIPBLG is shown in Figure 3, and observed and calculated reciprocal coordinates, observed intensities, and indices are given in Table II.

The observed density of *m*-CIPBLG was 1.356 g cm^{-3} , this value is larger than that calculated as 1.343 g cm^{-3} . A similar density anomaly has also been reported for poly(γ -methyl L-glutamate).⁸

p-CIPBLG

43 reflections with diffuse streaks were observed on the X-ray diffraction pattern of *p*-CIPBLG fibers which assume the $7/2$ α -helical conformation. The equatorial reflections can be indexed on a hexagonal net of $a=b=15.24 \text{ \AA}$. Though the row-line reciprocal coordinates of most reflections on the 2nd layer-line coincide with those of the equatorial reflections, other reflections cannot be indexed on the hexagonal unit cell. In order to index all reflections, an orthorhombic unit cell with $a=15.24 \text{ \AA}$ and $b=26.40 \text{ \AA}$ is adopted. This unit cell contains two molecules, and is shown in Figure 1c. All other layer-lines except for the equator have diffuse streaks. Sharp reflections with diffuse streaks are also observed on the 2nd layer-line. These observations are different from those for *o*-CIPBLG and *m*-CIPBLG, but similar to that for PBLG.⁹

The repeat distance along the fiber axis (*c*-axis) was 10.67 \AA . Figure 4 shows the reciprocal rotation lattice diagram of *p*-CIPBLG. Table III lists the observed and calculated values of reciprocal coordinates, observed intensities, and indices.

The observed density was 1.362 g cm^{-3} , compared to the calculated value of 1.371 g cm^{-3} .

Cylindrical Patterson Map

Takeda *et al.*² succeeded in locating the chlorine atom in *p*-CIPBLA using the cylindrical Patterson

Table II. X-Ray reflections of poly[γ -(*m*-chlorobenzyl) L-glutamate] fibers^a

| R_o | R_c | I_o | h | k | l | R_o | R_c | I_o | h | k | l |
|--------|--------|-------|-----|-----|-----|--------|--------|-------|-----|-----|-----|
| 0.0742 | 0.0745 | 441.7 | 1 | 0 | 0 | 0.3438 | 0.3413 | 2.6 | 4 | 1 | 3 |
| 0.1289 | 0.1290 | 284.1 | 1 | 1 | 0 | 0.3659 | 0.3724 | 1.8 | 5 | 0 | 3 |
| 0.1489 | 0.1490 | 329.0 | 2 | 0 | 0 | 0.3862 | 0.3870 | 3.0 | 3 | 3 | 3 |
| 0.1974 | 0.1971 | 192.4 | 2 | 1 | 0 | 0.3928 | 0.3941 | 3.0 | 4 | 2 | 3 |
| 0.2230 | 0.2234 | 3.3 | 3 | 0 | 0 | 0.4113 | 0.4147 | 2.6 | 5 | 1 | 3 |
| 0.2572 | 0.2580 | 126.6 | 2 | 2 | 0 | 0.0752 | 0.0745 | 7.3 | 1 | 0 | 5 |
| 0.2683 | 0.2685 | 323.4 | 3 | 1 | 0 | 0.1314 | 0.1290 | 61.8 | 1 | 1 | 5 |
| 0.2974 | 0.2979 | 99.6 | 4 | 0 | 0 | 0.1470 | 0.1490 | 110.2 | 2 | 0 | 5 |
| 0.3263 | 0.3247 | 103.1 | 3 | 2 | 0 | 0.2013 | 0.1971 | 154.4 | 2 | 1 | 5 |
| 0.2416 | 0.3413 | 60.9 | 4 | 1 | 0 | 0.2251 | 0.2245 | 34.8 | 3 | 0 | 5 |
| 0.3731 | 0.3724 | 4.2 | 5 | 0 | 0 | 0.2558 | 0.2580 | 3.0 | 1 | 1 | 5 |
| 0.3825 | 0.3870 | 2.4 | 3 | 3 | 0 | 0.2676 | 0.2685 | 3.1 | 3 | 1 | 5 |
| 0.3936 | 0.3941 | 5.9 | 4 | 2 | 0 | 0.2930 | 0.2979 | 0.7 | 4 | 0 | 5 |
| 0.4140 | 0.4147 | 4.5 | 5 | 1 | 0 | 0.3233 | 0.3247 | 1.4 | 3 | 2 | 5 |
| 0.4420 | 0.4469 | 2.2 | 6 | 0 | 0 | 0.3468 | 0.3413 | 2.8 | 4 | 1 | 5 |
| 0.2246 | 0.2234 | 13.8 | 3 | 0 | 1 | 0.3718 | 0.3724 | 1.2 | 5 | 0 | 5 |
| 0.1495 | 0.1490 | 0.7 | 2 | 0 | 2 | 0.3867 | 0.3870 | 2.4 | 3 | 3 | 5 |
| 0.1961 | 0.1971 | 66.5 | 2 | 1 | 2 | 0.3950 | 0.3941 | 2.4 | 4 | 2 | 5 |
| 0.2241 | 0.2234 | 26.5 | 3 | 0 | 2 | 0.4164 | 0.4147 | 1.4 | 5 | 1 | 5 |
| 0.2564 | 0.2580 | 18.6 | 2 | 2 | 2 | 0.0741 | 0.0745 | 3.0 | 1 | 0 | 8 |
| 0.2676 | 0.2685 | 16.8 | 3 | 1 | 2 | 0.1301 | 0.1290 | 5.5 | 1 | 1 | 8 |
| 0.0747 | 0.0745 | 1.7 | 1 | 0 | 3 | 0.1491 | 0.1490 | 8.0 | 2 | 0 | 8 |
| 0.1308 | 0.1290 | 4.7 | 1 | 1 | 3 | 0.1996 | 0.1971 | 10.9 | 2 | 1 | 8 |
| 0.1518 | 0.1490 | 18.0 | 2 | 0 | 3 | 0.2229 | 0.2234 | 4.8 | 3 | 0 | 8 |
| 0.1973 | 0.1971 | 16.9 | 2 | 1 | 3 | 0.2560 | 0.2685 | 4.2 | 3 | 1 | 8 |
| 0.2251 | 0.2234 | 0.7 | 3 | 0 | 3 | 0.0762 | 0.0745 | 6.8 | 1 | 0 | 10 |
| 0.2580 | 0.2580 | 0.9 | 2 | 2 | 3 | 0.1328 | 0.1290 | 7.5 | 1 | 1 | 10 |
| 0.2701 | 0.2685 | 1.1 | 3 | 1 | 3 | 0.2686 | 0.2685 | 5.7 | 3 | 1 | 10 |
| 0.2959 | 0.2979 | 1.3 | 4 | 0 | 3 | 0.2952 | 0.2979 | 3.6 | 4 | 0 | 10 |
| 0.3234 | 0.3247 | 2.6 | 3 | 2 | 3 | 0.0000 | 0.0000 | — | 0 | 0 | 18 |

^a Unit cell dimensions: $a=b=15.50 \text{ \AA}$ and $c=27.10 \text{ \AA}$.

map. We also attempted to locate this atom in *o*-CIPBLG, *m*-CIPBLG, and *p*-CIPBLG by the method of MacGillavry and Bruins.¹⁰

Figure 5 shows the cylindrical Patterson map for *o*-CIPBLG. The peaks observed in the region from $r=0$ to 4 \AA are those which may be expected for the α -helix structure. Peaks at about $r=15 \text{ \AA}$ correspond to the interchain vector between α -helices. Besides these, there are peaks at about $r=8 \text{ \AA}$, which may be assigned to the intra-chain chlorine-chlorine vector. These peaks are situated on the loop corresponding to a helix with a radius of 4 \AA and a pitch of 5.4 \AA .

Figure 6 shows the cylindrical Patterson map for *m*-CIPBLG. As in *o*-CIPBLG, peaks can be observed in the region from $r=0$ to 4.5 \AA and at $r=15 \text{ \AA}$. Peaks assignable to the chlorine-chlorine vector are

found at $r=7.5 \text{ \AA}$ and $r=12.5 \text{ \AA}$. These peaks are situated on the loop corresponding to a helix with a radius of 6.2 \AA and a pitch of 5.4 \AA .

Figure 7 illustrates the cylindrical Patterson map for *p*-CIPBLG. Peaks observed at 9 \AA correspond to the chlorine-chlorine vector, and are on the loop corresponding to a helix with a radius of about 4.5 \AA and a pitch of 10.7 \AA .

The fact that these peaks are found not on the loop for a helix with a radius of 4.5 \AA and a pitch of 5.35 \AA indicates that they do not correspond to the chlorine-chlorine vector in the same helix. Thus, we may infer that the chlorine atoms belonging to neighboring helices surround one helix at a distance of 4.5 \AA from the helix axis. On the other hand, the chlorine atom belonging to the same helix is located at a distance 10 \AA from the helix axis. The peak at

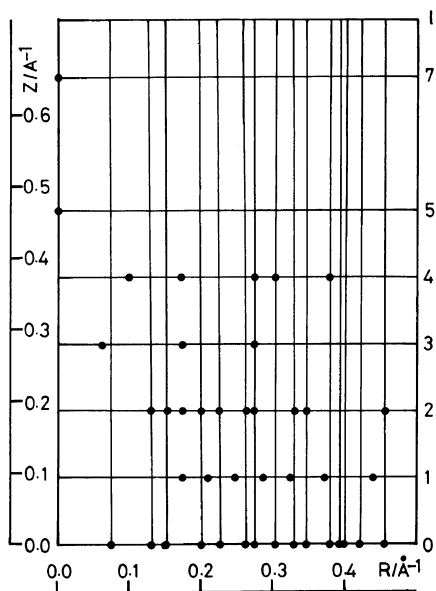


Figure 4. Reciprocal lattice rotation diagram of *p*-CIPBLG.

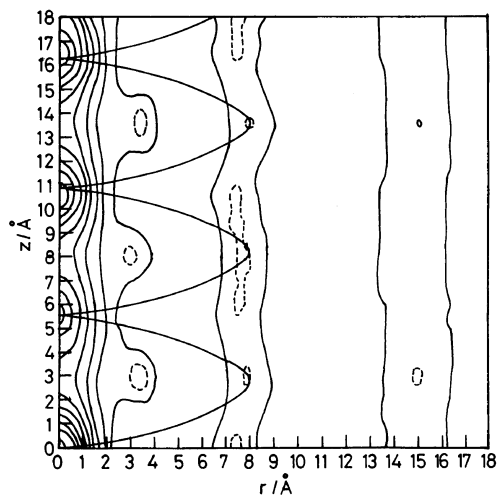


Figure 5. Cylindrical Patterson map of *o*-CIPBLG. Contours are drawn at arbitrary, equally separated levels.

Table III. X-Ray reflections of poly[γ -(*p*-chlorobenzyl) L-glutamate] fibers^a

| R_o | R_c | I_o | h | k | l | R_o | R_c | I_o | h | k | l |
|--------|--------------------|-------|----------|--------|--------|--------|--------------------|-------|-----------|--------|--------|
| 0.0757 | 0.0757 | 256.4 | 2 | 0 | 0 | 0.4407 | { 0.4367 0.4464 | 7.6 | { 11 8 | 2 5 | 1 1 |
| 0.1312 | 0.1312 | 107.2 | 3 | 1 | 0 | 0.1310 | 0.1312 | 120.4 | 3 | 1 | 2 |
| 0.1517 | 0.1515 | 215.4 | 4 | 0 | 0 | 0.1525 | 0.1515 | 85.5 | 4 | 0 | 2 |
| 0.2000 | 0.2004 | 459.5 | 5 | 1 | 0 | 0.1755 | 0.1735 | 39.4 | 3 | 2 | 2 |
| 0.2283 | 0.2273 | 480.0 | 6 | 0 | 0 | 0.2011 | 0.2004 | 37.6 | 5 | 1 | 2 |
| 0.2625 | 0.2625 | 195.1 | 6 | 2 | 0 | 0.2277 | 0.2273 | 42.8 | 6 | 0 | 2 |
| 0.2740 | 0.2732 | 203.5 | 7 | 1 | 0 | 0.2635 | 0.2625 | 10.8 | 6 | 2 | 2 |
| 0.3012 | 0.3030 | 53.5 | 8 | 0 | 0 | 0.2795 | 0.2732 | 11.8 | 7 | 1 | 2 |
| 0.3279 | 0.3300 | 33.6 | 8 | 2 | 1 | 0.3291 | 0.3300 | 5.5 | 8 | 2 | 2 |
| 0.3478 | 0.3472 | 5.7 | 9 | 1 | 0 | 0.3450 | 0.3472 | 12.4 | 9 | 1 | 2 |
| 0.3765 | 0.3787 | 6.6 | 10 | 0 | 0 | 0.4543 | 0.4542 | 14.4 | 12 | 0 | 2 |
| 0.3937 | 0.3937 | 3.6 | 9 | 3 | 0 | 0.0653 | 0.0656 | 42.3 | 0 | 1 | 3 |
| 0.4049 | 0.4016 | 10.5 | 10 | 2 | 0 | 0.1724 | 0.1735 | 62.2 | 3 | 2 | 3 |
| 0.4274 | 0.4219 | 7.5 | 11 | 1 | 0 | 0.2777 | 0.2732 | 22.5 | 7 | 1 | 3 |
| 0.4542 | 0.4542 | 2.9 | 12 | 0 | 0 | 0.4001 | 0.3989 | 19.8 | 6 | 5 | 3 |
| 0.1715 | 0.1735 | 61.7 | 3 | 2 | 1 | 0.1023 | 0.1002 | 9.0 | 2 | 1 | 4 |
| 0.2102 | 0.2109 | 82.0 | 2 | 3 | 1 | 0.1753 | 0.1735 | 9.5 | 3 | 2 | 4 |
| 0.2485 | 0.2483 | 38.8 | 4 | 3 | 1 | 0.2767 | 0.2730 | 4.0 | 7 | 1 | 4 |
| 0.2810 | 0.2859 | 27.5 | 3 | 4 | 1 | 0.3028 | 0.3029 | 2.6 | 8 | 0 | 4 |
| 0.3180 | 0.3235 | 23.2 | 5 | 4 | 1 | 0.3819 | 0.3765 | 3.9 | 9 | 1 | 4 |
| 0.3735 | { 0.3729 0.4365 | 8.3 | { 7 5 | 4 6 | 1 1 | 0.0000 | 0.0000 | — | 0 | 0 | 5 |
| | | | | | | 0.0000 | 0.0000 | — | 0 | 0 | 7 |

^a Unit cell dimensions: $a = 15.24 \text{ \AA}$, $b = 26.24 \text{ \AA}$, and $c = 10.67 \text{ \AA}$.

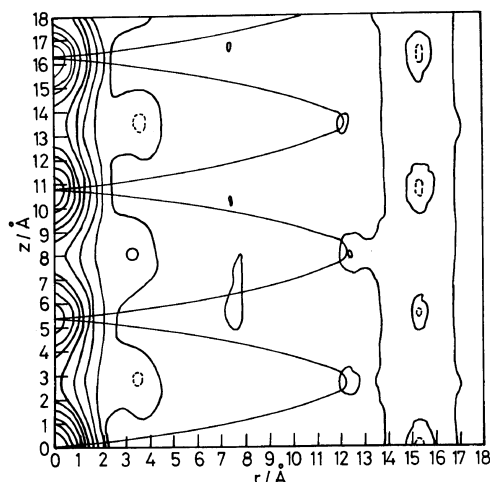


Figure 6. Cylindrical Patterson map of *m*-CIPBLG. Contours are drawn at arbitrary, equally separated levels.

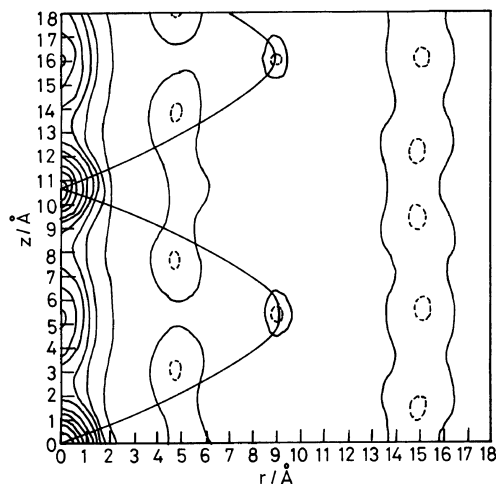


Figure 7. Cylindrical Patterson map of *p*-CIPBLG. Contours are drawn at arbitrary, equally separated levels.

about 5 Å along the fiber axis accounts for the chlorine-chlorine vector in the inter-helix region. In the cylindrical Patterson map for PBLG,¹¹ no peaks other than those due to the main chain were observed.

Equatorial Fourier Map

It is difficult to determine the crystal structure of the polymers studied in this work by the Fourier

synthesis because of the limited number of reflections and lack of symmetry. However, these polymers have chlorine atoms whose location we can expect to find. We evaluated the electron density map projected onto the (*ab*) plane by the equatorial Fourier synthesis. Equatorial reflections of *o*-CIPBLG and *m*-CIPBLG were indexed on hexagonal nets, and it was found that these polymers have an apparent 6-fold axis along the *c*-axis on the projection due to the 18/5 helix conformation. This indicates that these polymers may have a center of symmetry on 6-fold axis. Although the 7/2 helix of *p*-CIPBLG does not have the 6-fold axis along the helical axis on the projection, the equatorial reflections can be indexed on the hexagonal net. This may be attributed to the presence of translational and angular disorders of the helix and a slight deviation of the 7/2 helix from the 18/5 helix. We may thus consider that *p*-CIPBLG has a center of symmetry on the projection.

If the structure is centrosymmetric, the electron density is given by,

$$\rho(x, y, 0) = \pm \sum |F(h, k, 0)| \cos 2\pi(hx + ky) \quad (1)$$

Since the correct sign of $F(h, k, 0)$ is not known, all possible sign combinations must be examined. For a helical structure, the Fourier transform corresponding to the equatorial layer line can be calculated approximately by the equation:

$$F(h, k, 0) = \sum_j f_j J_0(2\pi R_{hk} r_j) \quad (2)$$

where R_{hk} (in \AA^{-1}) is the distance from the vertical axis in the reciprocal space, f_j , the atomic scattering factor, r_j , the distance of the j -th atom from the helix axis, and $J_0(x)$, the zero-th order Bessel function of x . We first determined the phase of $F(h, k, 0)$ from eq 2 using the radial distances of the main chain atoms of an α -helix and of the chlorine atom obtained by the cylindrical Patterson map. However, the equatorial Fourier maps obtained with this calculated phase were not consistent with the electron density expected from the main chain of an α -helix and the cylindrical Patterson maps. Thus, the procedure discussed in the following was used to find the correct phase.

If the number of observed equatorial reflections are fifteen, the possible sign combinations are $2^{14} = 16384$. Since this number is very large, only the relatively strong nine reflections (eight reflections for *o*-CIPBLG) were considered. This reduced the

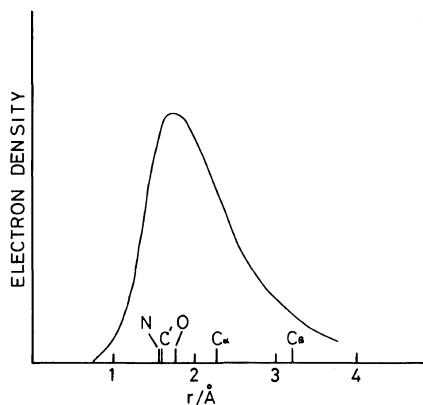


Figure 8. Electron density of radial section expected from the main chain of α -helix.¹²

sign to $2^8=256$. First, the electron density of the radial section was calculated in the range from 0 to 4 Å. At this stage, sign combinations giving electron densities incompatible with the α -helical main chain features as shown in Figure 8 were excluded. Secondly, the electron density of the radial section beyond 8 Å was calculated, and those that were reasonable, on the basis of the radial distance of the chlorine atom in the cylindrical Patterson map, were selected. Thirdly, the electron densities of a few selected combinations were calculated throughout a unit cell using all reflections. The most probable one was selected from these calculated electron densities.

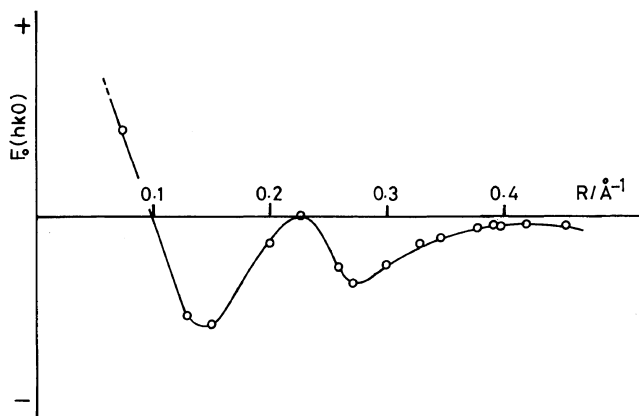


Figure 9. Observed amplitudes of the equatorial reflections of *o*-CIPBLG.

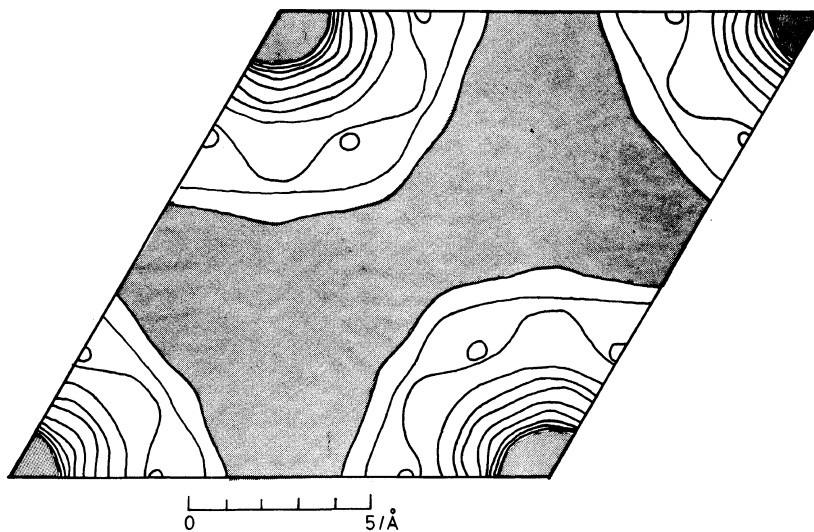


Figure 10. Equatorial Fourier map of *o*-CIPBLG. Contours are drawn at arbitrary, equally separated levels.

The set of signs of the structure factor for *o*-CIPBLG is shown in Figure 9 and the corresponding electron density map is displayed in Figure 10. The density distribution at the corner of the unit cell is as expected from the α -helical main chain. The peak at about 4 Å is due to the chlorine atom. Thus, the chlorine atom is located at a relatively small distance from the helix core, and may incur some steric hindrance.

The set of signs of the structure factor for *m*-CIPBLG is shown in Figure 11, with the corresponding electron density map given in Figure 12. The peak of the chlorine atom was found at about

6 Å from the helix axis, indicating that the chlorine atom of *m*-CIPBLG is situated between the two adjacent polymer molecule.

The set of signs of the electron density distribution for *p*-CIPBLG are shown in Figures 13 and 14, respectively. The assignment of a particular chlorine atom to the nearest neighboring molecule may be made on the basis of the peak at 5 Å. Thus, the radial distance of the chlorine atom from the helix axis is 10–10.5 Å which is in agreement with the cylindrical Patterson map.

The structural parameters are summarized in Table IV and indicate that substitution of a chlorine

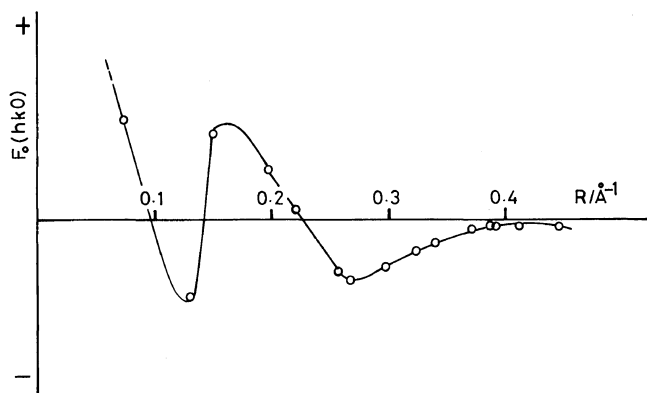


Figure 11. Observed amplitudes of the equatorial reflections of *m*-CIPBLG.

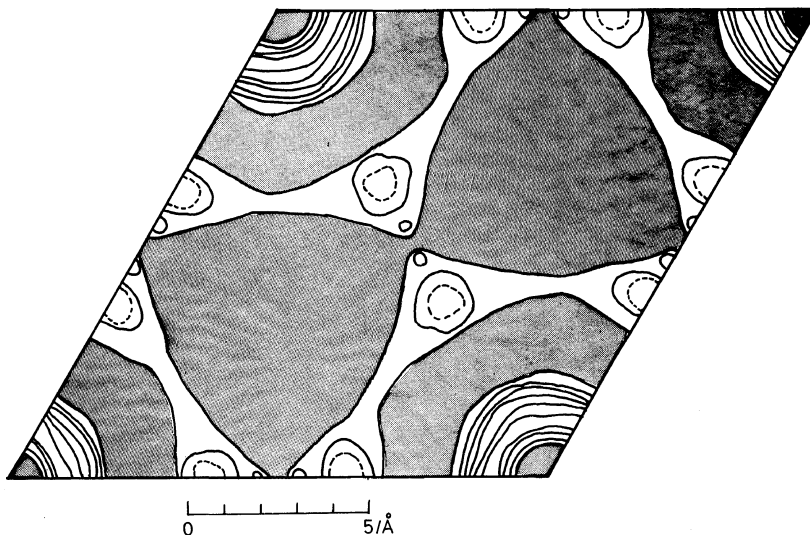


Figure 12. Equatorial Fourier map of *m*-CIPBLG. Contours are drawn at arbitrary, equally separated levels.

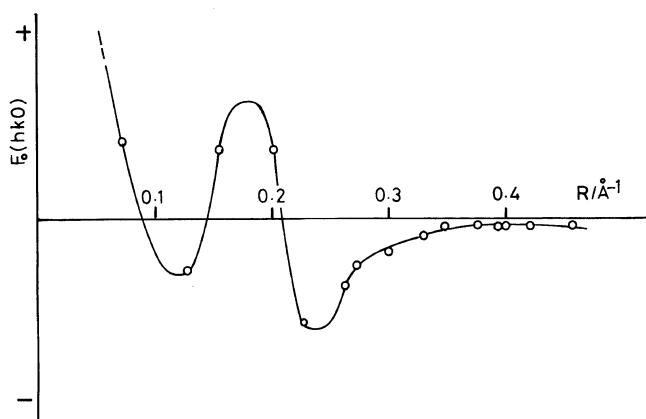


Figure 13. Observed amplitudes of the equatorial reflections of *p*-CIPBLG.

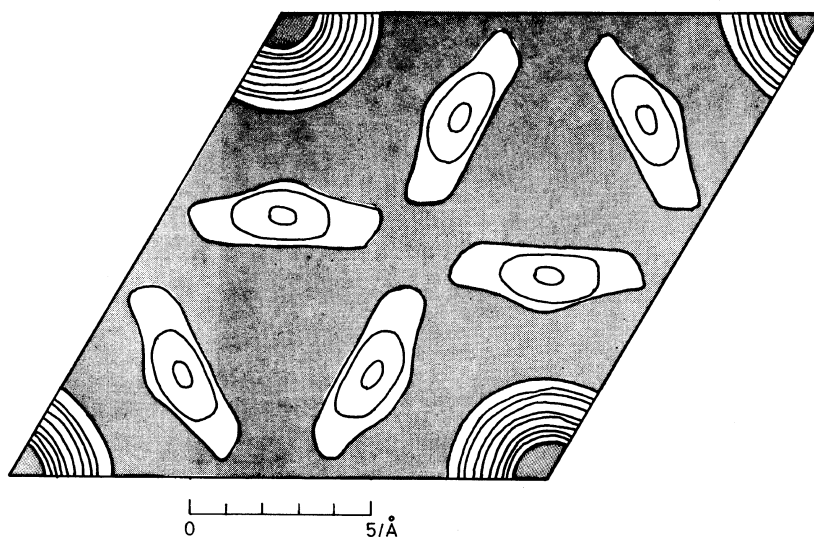


Figure 14. Equatorial Fourier map of *p*-CIPBLG. Contours are drawn at arbitrary, equally separated levels.

atom at the *o*-, or *m*-position of the phenyl ring of PBLG does not change the main chain conformation. On the contrary, substitution of a chlorine atom at the *p*-position of the phenyl ring of PBLG changes the main chain conformation. The distance between the nearest molecules and the helix parameters also vary with the difference in the substitutional position of the chlorine atom. The distance between the nearest chlorine substituted PBLG molecules is larger than that between unsubstituted PBLG. This difference may be interpreted in terms of the interaction between the side chains of neighboring molecules. Fraser *et al.*¹⁴ made X-

ray diffraction and infrared spectra studies on poly[γ -(*o*-nitrobenzyl) L-glutamate] (*o*-NO₂PBLG), poly[γ -(*m*-nitrobenzyl) L-glutamate] (*m*-NO₂PBLG), and poly[γ -(*p*-nitrobenzyl) L-glutamate] (*p*-NO₂PBLG) in the solid state, and suggested that, as our results also show, the outer portion of the side chain influences the geometry of the α -helical conformation, as illustrated in Table IV.

The distance of the chlorine atom from the helix axis was found to be about 4, 6, and 10 Å for *o*-, *m*-, and *p*-CIPBLG, respectively. If the side chain is oriented perpendicularly to the helix axis, these distances in *o*-CIPBLG, *m*-CIPBLG, and *p*-CIPBLG

Table IV. Comparison of intermolecular distances, helical parameters in α -helical conformations, and densities of poly[γ -(monochlorobenzyl) L-glutamate]s

| | Intermolecular distances | | Helix parameter ^d | | Density ^e | |
|---|--------------------------|-------------------|------------------------------|--------------------|----------------------|----------------------|
| | Å | Conformation | <i>P</i> | <i>p</i> | <i>d_c</i> | <i>d_o</i> |
| | | | Å | Å | g cm ⁻³ | g cm ⁻³ |
| PBLG | 14.95 ^a | 18/5 ^b | 5.42 ^b | 1.505 ^b | 1.260 ^a | 1.25 ^b |
| <i>o</i> -CIPBLG | 15.30 | 18/5 | 5.40 | 1.500 | 1.382 | 1.367 |
| <i>m</i> -CIPBLG | 15.50 | 18/5 | 5.42 | 1.505 | 1.343 | 1.356 |
| <i>p</i> -CIPBLG | 15.24 | 7/2 | 5.34 | 1.524 | 1.371 | 1.362 |
| <i>o</i> -NO ₂ PBLG ^c | — | — | 5.34 | 1.495 | — | — |
| <i>m</i> -NO ₂ PBLG ^c | — | — | 5.32 | 1.485 | — | — |
| <i>p</i> -NO ₂ PBLG ^c | — | — | 5.51 | 1.525 | — | — |

^a Reference 13.^b Reference 9.^c Reference 14.^d *P* is the pitch of the helix and *p*, the axial height per residue.^e *d_c* is the calculated density and *d_o*, the observed density.

should be about 8–10, 10–12, and 13 Å, respectively. However, the observed values for *o*- and *m*-CIPBLG are appreciably smaller than these values. Hence, it is likely that the side chains of these polypeptides are bent or deviate to some extent from the line perpendicular to the helix axis. Moreover, we inferred that the side chain conformations of *o*- and *m*-CIPBLG are similar to each other, while the side chain of *p*-CIPBLG is straight and perpendicular to the helix axis.

Sasaki *et al.*,¹⁵ based on their study of molecular motions in solid *o*-, *m*-, and *p*-CIPBDG by dielectric measurement suggest that (1) the molecular motions of the side chains of *o*-, *m*-, and *p*-CIPBDG are hindered more than those in PBLG; (2) the chlorine atom of *o*-CIPBDG is located at a relatively short distance from the helix axis; and (3) the chlorine atom of *p*-CIPBDG is probably located in the outermost part of the side chain. These results are consistent with our conclusions.

It is likely that the presence of a large unit cell containing a hexagonal unit cell as a subunit in *o*- and *p*-CIPBLG is due to molecular orientation primarily. However, there remains the possibility of the regular arrangement of up and down chains also inferred for the case of ω -helical *p*-CIPBLA.² It is also likely that the diffuse streaks appearing on the diffraction patterns of *o*-, *m*-, and *p*-CIPBLG fibers

result from the disorder of the helical sense and/or translational and angular displacements of the chains.

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REFERENCES

1. E. M. Bradbury, A. R. Downie, A. Elliott, and W. E. Hanby, *Proc. R. Soc. London, Ser. A*, **259**, 110 (1960).
2. Y. Takeda, Y. Iitaka, and M. Tsuboi, *J. Mol. Biol.*, **51**, 101 (1970).
3. M. Osanai and K. Hikichi, *Polym. J.*, **13**, 817 (1981).
4. M. Osanai and K. Hikichi, *Biopolymers*, **19**, 1099 (1980).
5. L. Brown and I. F. Trotter, *Trans. Faraday Soc.*, **52**, 537 (1956).
6. G. D. Fasman, Ed., "Poly- α -Amino Acids," Marcel Dekker, Inc., New York, N. Y., 1967, p 1.
7. F. Colonna-Cesari and S. Permilat, *Polymer*, **17**, 267 (1976).
8. C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby, and I. F. Trotter, *Proc. R. Soc. London, Ser. B*, **141**, 49 (1953).
9. A. Elliott, R. D. B. Fraser, and T. P. MacRae, *J. Mol. Biol.*, **11**, 821 (1965).
10. C. H. MacGillavry and E. M. Bruins, *Acta Cryst.*,

- 11, 199 (1967).
11. Y. Mitsui, Y. Iitaka, and M. Tsuboi, *J. Mol. Biol.*, **24**, 15 (1967).
 12. L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U.S.A.*, **37**, 235 (1951).
 13. C. H. Bamford, W. E. Hanby, and F. Happey, "Synthetic Polypeptides," Academic Press, New York, N.Y., 1956, p 267.
 14. R. D. B. Fraser, B. S. Harrap, R. Ledger, T. P. MacRae, F. H. C. Stewart, and E. Suzuki, *Biopolymers*, **5**, 797 (1967).
 15. N. Sasaki, A. Tsutsumi, K. Hikichi, Y. Konishi, and M. Hatano, *Polym. J.*, **11**, 583 (1979).