

X-Ray Scattering of Synthetic Poly(α -amino acid)s in the Solid State.

III. Temperature Dependence of the 1.5 Å-Meridional Reflection of the α -Helix

NORIO MATSUSHIMA, KUNIO HIKICHI, AKIHIRO TSUTSUMI,
and MOTOZO KANEKO

*Department of Polymer Science, Faculty of Science,
Hokkaido University, Sapporo 060, Japan.*

(Received August 4, 1975)

ABSTRACT: Spacing of the 1.5 Å-meridional reflection characteristic of the α -helical conformation and corresponding integrated intensity for poly(γ -benzyl D-glutamate), poly(γ -methyl D-glutamate), copoly(γ -benzyl L-glutamate, γ -methyl L-glutamate), and the racemic form of poly(γ -benzyl glutamate) were measured as a function of temperature from -90 to 120°C . Except for racemic poly(γ -benzyl glutamate), it was found that the spacing—temperature curve shows a discontinuity in slope in the vicinity of room temperature, which has been attributed to the onset of side-chain motion. This suggests that the transition is a crystalline phase transition of the second order. The linear thermal expansion coefficients of the 1.5 Å spacings for these polymers all were in order of magnitude of $10^{-5}/^\circ\text{C}$. It has been suggested that anharmonic vibration is affected by the nature of the side chain and by the side-chain motion. The intensity was found to decrease with increasing temperature, which was interpreted in terms of a Debye—Waller temperature factor for helical backbone atoms. From the temperature dependence of the 1.5 Å-reflection intensity, Young's modulus of the helix in the direction of the helical axis was estimated. Both the spacing and the intensity of racemic poly(γ -benzyl glutamate) were found to show a small but definite sudden change in the vicinity of 80°C . These indicate that the first-order phase transition at 80°C is accompanied by not only the breakdown of regular stacks of benzyl groups but also a sudden change in the mobility of the α -helical backbone.

KEY WORDS Poly(γ -benzyl glutamate) / Poly(γ -methyl D-glutamate) / Copoly(γ -benzyl L-glutamate, γ -methyl L-glutamate) / Transition / Young's Modulus / α -Helix /

There is a considerable interest in obtaining an understanding of properties of the α -helical conformation which poly(α -amino acid) adopts both in solution and in the solid state. The mobility of the α -helical backbone chain in the solid state and the effect of differing side chains upon the mobility have received less attention.

Molecular motions of many poly(α -amino acid)s have been widely investigated.¹⁻¹⁶ Nuclear magnetic resonance (NMR) studies on solid poly(γ -benzyl L-glutamate) (PBLG), poly(γ -methyl L-glutamate) (PMLG), poly(N^ϵ -carbobenzyloxy L-lysine), and other α -helical synthetic poly(α -amino acid)s showed that there occur appreciable changes in the second moment with temperature,

which have been accounted for wholly in terms of side-chain motions. Dynamic mechanical and dielectric measurements for these polymers also showed relaxations due to side-chain motions. All of these studies revealed that a considerable degree of freedom of side-chain motions is available at appropriate temperatures. But the α -helical backbone was regarded as to be rigid in the solid state.

The "standard" α -helical conformation consists of 18 residues in 5 turns in a repeat of 27 Å in the helical axis.^{17,18} Thus, in the X-ray diffraction pattern, there occurs a prominent meridional reflection with a spacing of 1.5 Å, which is an axial translation per amino acid.

The 1.5 Å-Reflection of the α -Helix

residue. Detailed investigation of the 1.5 Å-reflection is expected to give us information on the mobility of the α -helical backbone.

The purpose of the present paper is to report preliminary results of the temperature dependence of the spacing and intensity of the 1.5 Å-reflection for poly(γ -benzyl D-glutamate) (PBDG), poly(γ -methyl D-glutamate) (PMDG), and copoly-(γ -benzyl L-glutamate, γ -methyl L-glutamate) (PBMLG), and the racemic form of poly(γ -benzyl glutamate) containing equal parts of PBDG and PBLG. The results obtained will be discussed in terms of the mobility of the α -helical backbone and transitions of the first order and of the second order.

EXPERIMENTAL

The PBDG, PBLG, and PBMLG used in this study were prepared from the respective *N*-carboxy anhydrides in dioxane. The molecular weights of PBDG and PBLG were determined from their viscosities measured in *N,N*-dimethylformamide, and were 162,000 and 158,000, respectively. For PBMLG, which is a copolymer having 0.5 methyl glutamate (MG) in mole fraction, the molecular weight was not determined. The sample of PMDG was kindly supplied by Ajinomoto Co. Ltd., (Ajicoat A-200).

All samples used for X-ray measurements were in the form of oriented films 0.4–0.8 mm thick cast from solutions of helix supporting solvents. Oriented films of PBDG, PBMLG, and PBDG+PBLG (1:1), which is a mixture of equal parts by weights of PBDG and PBLG, were prepared by slow evaporation of concentrated methylene chloride solution in a magnetic field of 6.8, 7.8, and 9.0 KG, respectively. These films showed a quite good orientation in the direction of a magnetic field. An oriented film of PMDG was prepared by stroking concentrated chloroform solution in one direction until it dried on a glass plate, using another glass plate. All samples were dried at 120°C *in vacuo* for a day to remove residual solvents.

The X-ray photographs were obtained with a flat-plate camera using Cu-K α radiation, with the incident beam perpendicular to the film plane. The X-ray measurements were performed

using a Rigaku Denki Diffractometer equipped with a scintillation counter and a pulse height analyzer. Cu-K α radiation generated at 35 KV and 20 mA was used. In order to make the measurements more accurate, intensities were counted by a point-to-point technique for 100 sec at intervals of 0.05° in Bragg angle (θ) in the central region of the peak and at intervals of 0.1° in the vicinity of tails. More than 10,000 counts were detected for the counting period. To obtain the the 1.5 Å-meridional reflection profile, the symmetrical transmission technique was employed.¹⁹ The oriented films were set in such a way that the direction of orientation was perpendicular to the rotational axis of the diffractometer. The background curve was assumed to be a straight line drawn through the intensities of $2\theta=58$ and 66° . The peak position was defined as the center of the half-width. No correction was made to the peak position. Only the relative change in peak position with temperature was measured. The temperature was changed from -90 to 120°C and measured by a copper—constantan thermocouple.

RESULTS AND DISCUSSION

Transition of the Second Order in PBDG, PMDG, and PBMLG

Figure 1 shows a flat-plate X-ray photograph of PMDG which was obtained by inclining the orientation axis to the beam at 31° . The 1.5 Å-meridional reflection which is characteristic of α -helical conformation is observed on the photograph. Reported values of the 1.5 Å spacing are slightly different for each poly(α -amino acid). For example, PMLG gives a preferred value of 1.497 Å.²⁰ The value for PBLG examined hitherto (in the solid state and in solution) lies between 1.495 and 1.510 Å.^{21–23} No effort was made to evaluate the difference in the spacing among samples in this work, because it is difficult to obtain an accurate value of the spacing.

Figure 2 shows the diffraction profiles of the 1.5 Å-meridional reflection scanned in the meridional direction for PBDG, PMDG, PBMLG, and PBDG+PBLG (1:1) at room temperature. By means of this technique we could measure

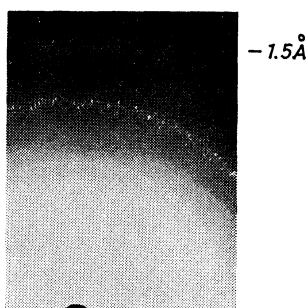


Figure 1. X-ray diffraction photograph of PMDG. Orientation axis is tilted 31° from the normal to the beam.

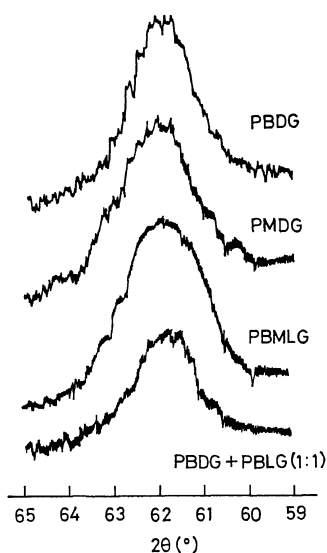


Figure 2. Diffraction profiles of the 1.5 Å-meridional reflection scanned in the meridional direction for PBDG, PMDG, PBMLG, and PBDG + PBLG (1:1). 2θ indicates scattering angle.

for the first time changes in spacing and intensity of the 1.5 Å-reflection with temperature.

Figure 3 shows the spacings of the 1.5 Å-reflection for PBDG, PMDG, and PBMLG as a function of temperature. For PMDG, the spacing almost linearly increases with increasing temperature from -70 to 0°C, where an abrupt change in slope occurs. Likewise, the spacings of the 1.5 Å-reflection for PBDG and PBMLG have break points in the vicinity of room temperature. Such break points have also been found in the temperature dependence of the lateral spacings between helices for PBLG and

PMLG in same temperature regions by the present authors.²⁴ It is therefore reasonable to assume that the abrupt increase in slope of the 1.5 Å spacings results from the same origin as that of the lateral spacings, which were interpreted in terms of the onset of side-chain motion. Since only the crystalline phase is responsible for Bragg reflection such as the 1.5 Å-meridional reflection, the present findings indicate that there exists a crystalline phase transition of the second order in PBDG, PMDG, and PBMLG in the vicinity of room temperature.^{24,25}

The linear thermal expansion coefficients of the 1.5 Å spacings for these polymers, α , are of the order of magnitude of $10^{-5}/^{\circ}\text{C}$, as listed in Table I, in which α_1 and α_2 refer to values above and below the transition temperature (T_2), respectively. These are one-tenth as large as those of the lateral spacings.²⁴ The values obtained are compared with that of ice, which assumes a typical net structure of the hydrogen bonds: $1\sim 6 \times 10^{-5}/^{\circ}\text{C}$ in a temperature range from -180 to -10°C .²⁶

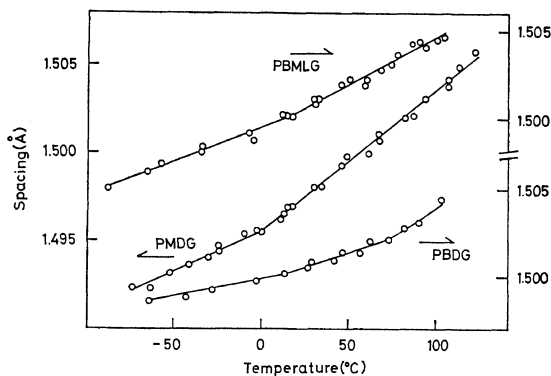


Figure 3. Spacings of the 1.5 Å-reflection for PBDG, PMDG, and PBMLG as a function of temperature.

Table I. Linear thermal expansion coefficients in the axial direction of PBDG, PBMLG, and PMDG

	$\alpha_1(T > T_2), ^{\circ}\text{C}^{-1}$	$\alpha_2(T < T_2), ^{\circ}\text{C}^{-1}$
PBDG	2.2×10^{-5}	1.4×10^{-5}
PBMLG	3.5×10^{-5}	2.4×10^{-5}
PMDG	5.4×10^{-5}	3.0×10^{-5}

T_2 , transition point at low temperatures.

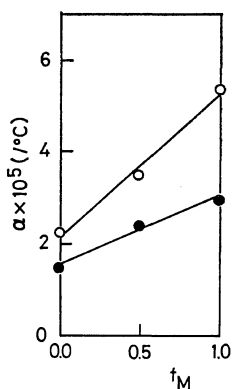


Figure 4. Linear thermal expansion coefficients in the axial direction, α , for copoly(γ -benzyl L-glutamate, γ -methyl L-glutamate) as a function of mole fraction of methyl glutamate f_M . Data for $f_M=0.0$ and 1.0 are of *D* polymers: ●, low temperature region; ○, high temperature region.

In Figure 4, the linear thermal expansion coefficients of the 1.5 Å spacings are plotted against mole fraction of MG (f_M). The expansion coefficients of the 1.5 Å spacings show a monotonous increase with increasing f_M both below and above the transition temperature. Although only one copolymer was investigated here, it seems evident that the inherent character of the individual side-chain and the composition in copolymer have some influence upon the thermal expansion of the α -helix in the axial direction.

Thus, the results obtained here suggest that anharmonic vibration of the α -helix in the direction of the helical axis is affected by the nature of the side chain and by the side-chain motion.

On the basis of results of optical rotation dispersion measurements of the helix-coil transition in copoly(γ -methyl L-glutamate, γ -benzyl L-glutamate), Roig and Cortijo²⁷ suggested that the stability of PMLG helix is less than that of PBLG and the stabilities in the copolypeptides are intermediate. These results were recently confirmed by high resolution NMR investigation in our laboratory.²⁸ If we assume a helix with a smaller thermal expansion coefficient in the axial direction to be a more stable helix, the present findings in the solid state are in close agreement with the results in solution.

The integrated intensity of the 1.5 Å-meridional reflection for PBDG, PMDG, and PBMLG was measured as a function of temperature. The intensities decreased with increasing temperature, in contrast with the intensities of the lateral reflections, which increased with temperature as reported in a previous paper.²⁴

The structure factor of the 1.5 Å-(0018) meridional reflection of the α -helix, $F(0018)$, is calculated by the equation

$$F(0018) = \sum_j f_j \exp(2\pi i z_j / d) \quad (1)$$

where d is the axial translation of an amino acid residue, z_j is the z -coordinate of atom j , and f_j is the atomic form factor, with the summation taken over all atoms in a residue. From eq 1 the absolute value of the structure factor for the 1.5 Å-reflection of the α -helix is calculated to be 6.1. In this calculation we did not take into account the contribution from side-chain atoms except for β -carbon nor the temperature effect. The atomic coordinates of 18/5 helix given by Pauling and Corey were used.²⁹ The measured value for PMLG was reported to be 5.8 by Brown and Trotter.¹⁷ This fact appears to indicate that the contribution to the observed intensity from the side chains is negligibly small.

Mitsui, *et al.*,³⁰ reported a possible side-chain conformation of PBDG+PBLG. Their results indicate that the contribution from the helical core and the side chains to the structure factors of the 1.5 Å-reflection, F_{core} and F_{side} , respectively, are opposite in sign and that $|F_{\text{core}}| > |F_{\text{side}}|$. If we assume that the Debye-Waller temperature factors of the side chains are the dominant factors, the observed intensity should increase with increasing temperature. This is not the case in the present work. Mitsui, *et al.*, have also reported the temperature factors of each atom at room temperature. If we take account of the temperature factors, the contribution from the side chain to the observed intensity is negligibly small. Therefore, we consider that the observed intensity is contributed from the helical backbone and that the observed temperature dependence is due to the temperature factor of the backbone atoms.

If we assume that the mean square of displacement along the axial direction, $\langle \Delta z^2 \rangle$, is same for all atoms, the intensity of the 1.5 Å-reflection can be written

$$I = I_0 \exp(-4\pi^2 \langle \Delta z^2 \rangle / d^2) \quad (2)$$

where I_0 is the intensity without any displacement and d is 1.5 Å.

Logarithms of the integrated intensities, as measured in units of the intensity at reference temperature T_0 , $\ln(I/I_0)$, were plotted against temperature in Figures 5, 6, and 7, for PBDG, PMDG, and PBMLG, respectively. T_0 was arbitrarily chosen to be the lowest temperature studied. It appears that $\ln(I/I_0)$ decreases linearly with increasing temperature. Moreover, break points are observed for PBDG and PBMLG, as seen in Figures 5 and 7. The break-point temperature for the intensity of PBDG is close to that for the spacing. Therefore, it probably comes from the same origin.

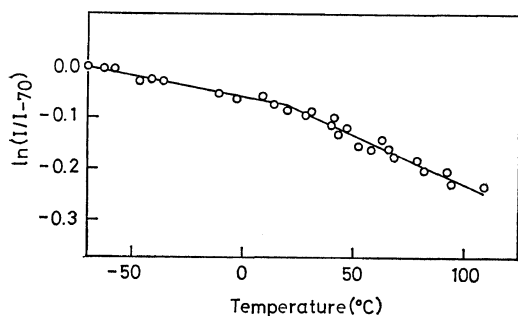


Figure 5. Logarithm of the relative intensity of the 1.5 Å-reflection for PBDG as a function of temperature.

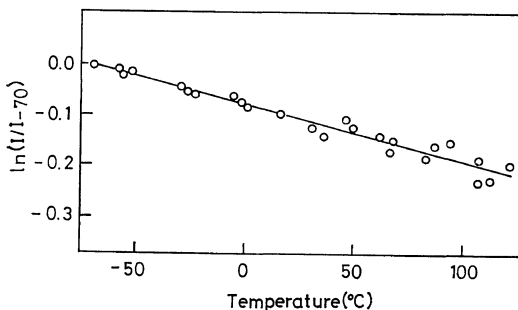


Figure 6. Logarithm of the relative intensity of the 1.5 Å-reflection for PMDG as a function of temperature.

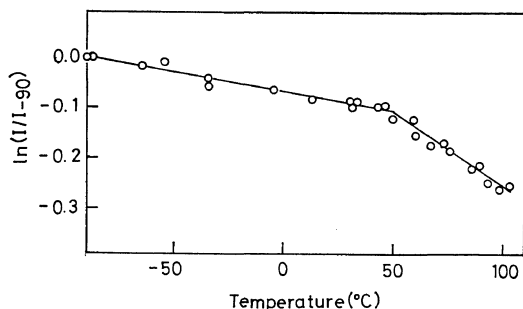


Figure 7. Logarithm of the relative intensity of the 1.5 Å-reflection for PBMLG as a function of temperature.

However, for PBMLG the break-point temperature for the intensity is a little higher than that for the spacing, and for PMDG the break was not observed. We will not discuss further about the break point for intensity, because we have not studied it in detail.

From the slopes in Figures 5, 6, and 7 we can estimate Young's modulus E of the helix in the direction of the helical axis. Assuming the classical harmonic approximation, the mean square displacement $\langle \Delta z^2 \rangle$ may be written as

$$\langle \Delta z^2 \rangle = kT/f \quad (3)$$

where k is Boltzmann's constant and f is the force constant for the stretching in the helical axis. Using f , Young's modulus E is given by

$$E = df/A \quad (4)$$

where A is the molecular cross section. Values of A for PBDG, PMDG, and PBMLG are 192, 123, and 175 Å², respectively.¹⁷ The value of PBMLG was estimated on the assumption of a hexagonal unit cell. From eq 2, 3, and 4, the logarithm of relative intensity is written as follows

$$\ln(I/I_0) = -(4\pi^2 k/AdE)(T - T_0) \quad (5)$$

Values of E estimated from the slope at low temperatures 2.5×10^{10} , 2.5×10^{10} , and 2.7×10^{10} dyn/cm² for PBDG, PBMLG, and PMDG, respectively. These values were compared with the value obtained from dynamic mechanical measurements: oriented PBLG film was reported to give a storage tensile modulus in the direction of orientation of 2.0×10^{10} dyn/cm².³¹ Itoh

and Shimanouchi calculated Young's modulus for poly L-alanine (PLA) from frequencies of accordion vibration and gave a value of 2.31×10^{11} dyn/cm².³² The cross section of PLA is much smaller than those of PBDG, PMDG, and PBMLG. Therefore, the observed values are thought to be in the correct order of magnitude.

Transition of the First Order in PBDG+PBLG (1:1)

Mixtures of PBDG and PBLG have been reported to assume the α -helix with 43 residues in 12 turns, although a strong layer line of 10.6 Å and the other weaker lines not expected from the α -helix occur in the diffraction patterns.^{30,33} Squire and Elliott suggested that these layer lines are caused by regular stacks of benzyl groups from about five side chains, which are formed by interaction between adjacent molecules of opposite screw sense. The phase transition of the first order was reported to occur in the vicinity of 80°C, which has been interpreted in terms of breakdown of the stacks.^{15,16,34}

Figure 8 shows the spacing of the 1.5 Å-reflection of PBDG+PBLG (1:1) as a function of temperature. There occurs a small but definite sudden increase in spacing of the 1.5 Å-reflection (about 0.4%) in the vicinity of 80°C, where the lateral spacings between helices also change abruptly.³⁴ The results obtained indicate that the phase transition near 80°C is reflected in the variation of the spacing of the 1.5 Å-meridional reflection as well. The amount of change in spacing of the 1.5 Å-reflection is about ten times smaller than that of the lateral spacings. The change in molar volume ΔV at the

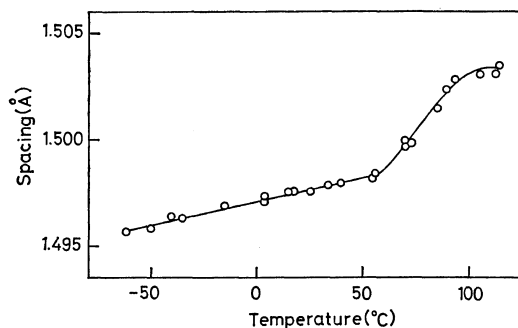


Figure 8. Spacing of the 1.5 Å-reflection for PBDG+PBLG (1:1) as a function of temperature.

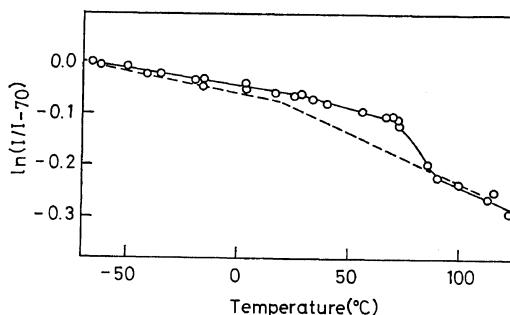


Figure 9. Logarithm of the relative intensity of the 1.5 Å-reflection for PBDG+PBLG (1:1) as a function of temperature. Dotted line indicates the data of PBDG.

transition temperature $T_1 \approx 80^\circ\text{C}$ was estimated to be $1.4 \text{ cm}^3/\text{mole}$, using data in a previous paper. The value is in agreement with $\Delta V = 1.8 \text{ cm}^3/\text{mole}$ obtained from dilatometric measurements.¹⁵ The linear thermal expansion coefficient obtained in the axial direction was nearly equal to that of PBDG, *i.e.*, $1.4 \times 10^{-5}/^\circ\text{C}$ below 80°C , although the strong interaction of side chains brings greater distortion of the helix.

Figure 9 shows the logarithm of the relative intensity of the 1.5 Å-reflection vs. temperature plots, the relative intensity being measured in units of the intensity at -70°C . The results for PBDG are also shown in the figure by the dotted line. The relative intensity of PBDG+PBLG (1:1) decreases with increasing temperature with slightly smaller slope than PBDG, with a faint indication of a break point at room temperature. It was found that the relative intensity suddenly decreases in the vicinity of 80°C to coincide with that of PBDG, corresponding to the sudden increase in spacing at the same temperature. These results indicate that below the transition temperature T_1 the thermal vibration of the helix is more restricted by the stacking of benzyl groups, and that the restriction is removed above T_1 . Wilser and Fitchen³⁵ reported that several small but definite Raman spectral changes appear when PBDG and PBLG are mixed, reaching a maximum for the racemic 50:50 mixture, and that the changes are removed reversibly above the transition temperature. This is likely to correspond to the results obtained in this work.

From eq 5, using the value of the slope in the intensity—temperature curve in Figure 9 at low temperature, Young's modulus in the axial direction is estimated to be 2.7×10^{10} dyn/cm².

CONCLUSION

The results obtained in this study suggest that the transition in the vicinity of room temperature for PBDG, PMDG, and PBMLG is a crystalline phase transition of the second order. The thermal expansion coefficients of the α -helices in the direction of the helical axis are all of the order of magnitude of $10^{-5}/^{\circ}\text{C}$ in the temperature range studied. It was suggested that anharmonic vibration is affected by the nature of the side chain and by the side-chain motion. The decrease in intensity of the 1.5 Å-reflection with increasing temperature was explained in terms of the temperature factors of the helical backbone atoms. Young's modulus of the helix was estimated from the temperature dependence of the 1.5 Å-reflection intensity. The first-order phase transition for PBDG + PBLG (1 : 1) in the vicinity of 80°C is accompanied by the sudden change in the mobility of the α -helical backbone.

Acknowledgments. The authors wish to express their gratitude to Professor J. Noguchi and Dr. S. Tokura of Hokkaido University for valuable advice in preparing samples, and Ajinomoto Co. Ltd., for supplying the poly(γ -methyl D-glutamate) sample. Thanks are also due to Professor T. Miyazawa of Tokyo University and Dr. K. Itoh of Waseda University for helpful comments. This work was supported by Ministry of Education, Japan.

REFERENCES

- J. A. E. Kail, J. A. Sauer, and A. E. Woodward, *J. Phys. Chem.*, **66**, 1292 (1962).
- K. Hikichi, *J. Phys. Soc. Japan*, **19**, 2167 (1964).
- A. Tsutsumi, *Japanese J. Appl. Phys.*, **9**, 1130 (1970).
- S. Ogawa, H. Obata, and T. Hatakeyama, Proc. 5th Intl. Congr. Rheol. Vol. III, S. Onogi, Ed., University of Tokyo Press, Tokyo, 1970, p 385.
- F. Happel, D. W. Jones, and B. W. Watson, *Biopolymers*, **10**, 2039 (1971).
- R. G. Saba, J. A. Sauer, and A. E. Woodward, *J. Polymer Sci., Part A*, **1**, 1483 (1963).
- K. Hikichi, K. Saito, M. Kakeko, and J. Furuichi, *J. Phys. Soc. Japan*, **19**, 577 (1964).
- S. Makino, K. Kamashima, S. Kubota, and S. Sugai, *Japanese J. Appl. Phys.*, **3**, 55 (1964).
- S. Sugai, K. Kamashima, S. Makino, and J. Noguchi, *J. Polym. Sci., Part A-2*, **4**, 183 (1966).
- J. V. Koleske and R. D. Lundberg, *Macromolecules*, **2**, 438 (1969).
- A. Hiltner, J. M. Anderson, and E. Borkowski, *ibid.*, **5**, 446 (1971).
- A. Tsutsumi, K. Hikichi, T. Takahashi, Y. Yamashita, N. Matsushima, K. Kanke, and M. Kaneko, *J. Macromol. Sci.-Phys.*, **B8**, 413 (1973).
- A. Hiltner, J. M. Anderson, and E. Bear, *ibid.*, **8**, 431, (1973).
- Y. Tsujita and I. Uematsu, *Polymer J.*, **6**, 274 (1974).
- T. Fukuzawa, I. Uematsu, and Y. Uematsu, *ibid.*, **6**, 537 (1974).
- T. Takahashi, A. Tsutsumi, K. Hikichi, and M. Kaneko, *Macromolecules*, **7**, 806 (1974).
- C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N.Y., 1956, Chapters 7 and 8.
- G. D. Fasman, "Poly- α -amino Acids," Marcel Dekker, Inc., New York, N.Y., 1967.
- L. E. Alexander, "X-ray Diffraction Methods in Polymer Science," John Wiley & Sons, Inc., New York, N. Y., 1969, p 78.
- C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby, and I. F. Trotter, *Proc. Roy. Soc.*, **B141**, 49 (1953).
- A. Elliott, R. D. B. Fraser, and T. P. MacRae, *J. Mol. Biol.*, **11**, 821 (1965).
- D. A. D. Parry and A. Elliott, *ibid.*, **25**, 1 (1967).
- J. M. Squire and A. Elliott, *Mol. Cryst. Liquid Cryst.*, **7**, 821 (1969).
- N. Matsushima, K. Hikichi, A. Tsutsumi, and M. Kaneko, *Polymer J.*, **7**, 44 (1975).
- K. Hikichi, A. Tsutsumi, S. Isozaki, and M. Kaneko, *ibid.*, **7**, 646 (1975).
- D. E. Eisenberg and W. Kauzman, "The Structure and Properties of Water," Oxford University Press, Ely House, London W.1. 1969, p 104.
- A. Roig and M. Cortijo, *Biopolymers*, **10**, 321 (1971).
- T. Hiraoki, A. Tsutsumi, K. Hikichi, and M. Kaneko, *Rep. Progr. Polym. Phys. Japan*, **17**, 575 (1974).

The 1.5 Å-Reflection of the α -Helix

29. L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 4241 (1951).
30. Y. Mitsui, Y. Iitaka, and M. Tsuboi, *J. Mol. Biol.*, **24**, 15 (1967).
31. S. Kotoda, K. Takagi, Y. Uematsu, and I. Uematsu, *Rep. Progr. Polym. Phys. Japan*, **15**, 629 (1972).
32. K. Itoh and T. Shimanouchi, *Biopolymers*, **9**, 383 (1970).
33. J. M. Squire and A. Elliott, *J. Mol. Biol.*, **65**, 291 (1972).
34. N. Matsushima, K. Hikichi, A. Tsutsumi, and M. Kaneko, *Polymer J.*, **7**, 382 (1975).
35. W. T. Wilser and D. B. Fitchen, *Biopolymers*, **13**, 1435 (1974).