

X-Ray Scattering of Synthetic Poly(α -amino acid)s in the Solid State. V. An Analysis of Poly(γ -methyl L-glutamate) and Poly(γ -benzyl L-glutamate) by a Coaxial Two Phase Model of Diffraction Intensity

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(Received November 21, 1977)

ABSTRACT: A coaxial two phase model is presented to account for the change with temperature in the intensity of the (100) reflection for the poly(γ -methyl L-glutamate) crystal and that of the (1 $\bar{1}$ 0) reflection for the poly(γ -benzyl L-glutamate) crystal. This model consists of two coaxial rods, whose central part represents a rigid helical backbone and the surrounding part, soft side chains. The equation for the temperature dependence of the ($hk0$) reflection intensity from the crystal in which the coaxial rods are hexagonally packed, is derived, and applied successfully to analyze the experimental results.

KEY WORDS Synthetic Polypeptide / α -Helix / Coaxial Two Phase Model / X-Ray Intensity / Temperature Dependence /

In the first paper of this series, we reported that the intensity of the (100) reflection for the poly(γ -methyl L-glutamate) (PMLG) crystal, and that of the (1 $\bar{1}$ 0) reflection for the poly(γ -benzyl L-glutamate) (PBLG) crystal increase with increasing temperature, and that these intensities are related to the lateral spacings between α -helices (hereafter referred to as lateral reflections). Furthermore, the square root of the intensity was found to be a linear function of the temperature (Figure 1).¹

A qualitative account for this finding may be found in the differences between the temperature factors of the atoms of the main-chain backbone and those of the side chains. Such a quantitative account may require a detailed structure analysis at each temperature, and this would be quite impracticable.

In order to account for this finding, we have adopted a sandwich two phase model which has been proposed to account for the results of small-angle X-ray scattering of crystalline polymers.² We have assumed that the rigid helical core corresponds to the crystalline parts, and the side-chain region to the amorphous surface layers. The results obtained were explainable by this sand-

wich two phase model. However, the sandwich two phase model is not satisfactory for describing the structures of α -helical PMLG and PBLG, as they are very different from that of a sandwich.

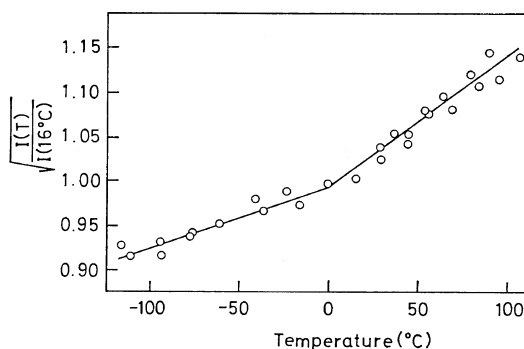


Figure 1. The relative change of the square root of the (100) Bragg reflection intensity of PMLG as a function of temperature by N. Matsushima, *et al.*¹

In this paper, we propose a more realistic model for PMLG and PBLG crystals and derive an equation for temperature dependence of the intensity of the lateral reflection on the basis of this new model.

A MODEL FOR ESTERS OF
POLY(GLUTAMIC ACID)

Figure 2 illustrates a model of PMLG and PBLG crystals. The center of the rod is a helical backbone with side-chains surrounding it. Since the helical backbone is regarded as rigid due to the intramolecular hydrogen bonds and the side chains long and flexible, we can assume that the center of the rod is more rigid and more dense than its surroundings. We call this the coaxial two phase model.

For the PMLG crystal the coaxial rods are closely arranged in a hexagonal net.³ The hexagonal arrangement is also approximately correct for

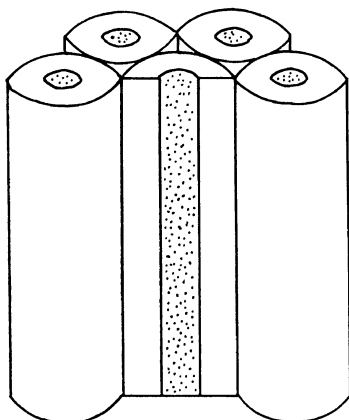


Figure 2. Coaxial two phase model of PMLG and PBLG: \blacksquare , rigid helical core; \square , soft side-chain region.

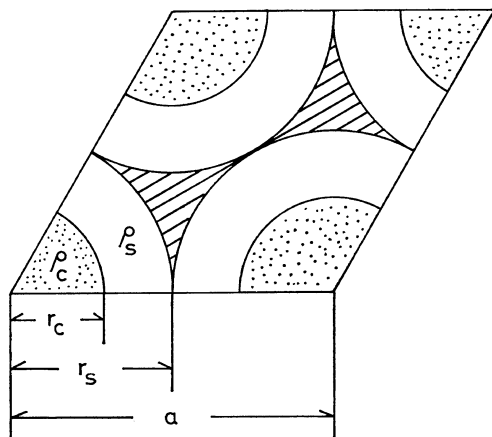


Figure 3. Unit cell of crystal composed of the two coaxial rods.

PBLG crystal.⁴ Figure 3 shows the projection of the unit cell on the plane perpendicular to the helical axis, where a is the lattice parameter, r_c and r_s are the radii of the main-chain helix and that of the total helix, respectively, and ρ_c and ρ_s are the electron density of the helical core and that of the side-chain region, respectively. We will calculate the diffraction intensity of the lateral reflection based on this model.

STRUCTURE FACTOR OF LATERAL
REFLECTION

To calculate the structure factor of the lateral reflection, one must recall that the reflection observed is a Bragg reflection which satisfies Laue equation. Taking account of this point, the structure factor $F(R)$ of the lateral reflection then may be written as

$$F(R) = \iint \rho(r) \exp(2\pi i R r \cos \theta) r \, dr \, d\theta \quad (1)$$

where R is the radial distance in the cylindrical reciprocal space, r , the radial distance from the center of the coaxial rod, and $\rho(r)$, the electron density projected to the plane perpendicular to the rod which is assumed to depend only on r ; $r \, dr \, d\theta$ is the element of area in the lateral plane, and θ is the polar angle in the lateral plane. The integration is performed over a unit cell.

For the coaxial two phase model we assumed that the electron density $\rho(r)$ is given by

$$\rho(r) = \begin{cases} \rho_c & \text{for } 0 \leq r \leq r_c \\ \rho_s & \text{for } r_c \leq r \leq r_s \end{cases} \quad (2)$$

Thus, the integration is reduced from 0 to 2π and r from 0 to r_s , because the hatched area of Figure 3 does not contribute to the integration.

Now, since

$$\int_0^{2\pi} \exp(2\pi i R r \cos \theta) \, d\theta = 2\pi J_0(2\pi R r) \quad (3)$$

Equation 1 becomes

$$F(R) = 2\pi \int_0^{r_s} \rho(r) J_0(2\pi R r) r \, dr \quad (4)$$

where J_0 is the Bessel function of the first kind of the zeroth order. Substituting eq 2 in eq 4 and using the identity

$$\int_0^r r J_0(2\pi R r) \, dr = \frac{r}{2\pi R} J_1(2\pi R r) \quad (5)$$

we obtain

$$F(R) = (\rho_c - \rho_s) \frac{r_c J_1(2\pi R r_c)}{R} + \rho_s \frac{r_s J_1(2\pi R r_s)}{R} \quad (6)$$

where J_1 is the Bessel function of the first order. The intensity is then given by the square of the structure factor $F(R)$. On the basis of eq 6, we can calculate the temperature-dependence of the lateral reflection intensity.

TEMPERATURE-DEPENDENCE OF THE INTENSITY OF LATERAL REFLECTIONS

Since all of the parameters, ρ_c , ρ_s , r_c , r_s , R and J_1 in eq 6 change with temperature, temperature-dependence for these parameters was derived at first. Since the electron density is thought to be proportional to the mass density, the temperature dependence of ρ_c and ρ_s are given by the following equations,

$$\frac{1}{\rho_c} = \frac{1}{\rho_{c0}} \{1 + 3\alpha_c(T - T_0)\} \quad (7)$$

$$\frac{1}{\rho_s} = \frac{1}{\rho_{s0}} \{1 + (2\alpha_s + \alpha_c)(T - T_0)\} \quad (8)$$

where α_c and α_s are the linear thermal expansion coefficients of the helical core and of the side-chain region, respectively. In eq 7 and 8 we assumed that the thermal expansion of the helical core is isotropic, and that the linear thermal expansion coefficient of the side-chain region in the direction of the helical axis is equal to that of the helical core. Here and in subsequent cases, the subscript 0 will represent the respective value

at temperature T_0 except for that of the Bessel function J_0 . As a matter of course, the corresponding equation for r_c is

$$r_c = r_{c0} \{1 + \alpha_c(T - T_0)\} \quad (9)$$

Since the unit cell of the crystal is a hexagonal lattice, it immediately follows

$$r_s = \frac{a}{2} \quad (10)$$

$$\frac{1}{R} = \left\{ \frac{3}{4(h^2 + hk + k^2)} \right\}^{1/2} a = ma \quad (11)$$

where h and k are the Miller indices. Thus both R and r_s are related to a . For the temperature dependence of a , we have

$$a = a_0 \left[1 + \left\{ \left(1 - \frac{2r_{c0}}{a_0} \right) \alpha_s + \frac{2r_{c0}}{a_0} \alpha_c \right\} (T - T_0) \right] \quad (12)$$

The Bessel function J_1 is a function of r_c and a . Thus, it also depends on temperature. To calculate the temperature-dependence of the Bessel function $J_1(x)$, we carried out Taylor's expansion using the value x_0 at temperature T_0 for $J_1(x)$. Since α_c and α_s are considered to be small, we have for $J_1(x)$.

$$J_1(x) = J_1(x_0) + \left\{ J_0(x_0) - \frac{J_1(x_0)}{x_0} \right\} (x - x_0) \quad (13)$$

Taking into account eq 6 to 13, we finally obtain,

$$\left\{ \frac{I(T)}{I(T_0)} \right\}^{1/2} = 1 + (A\alpha_s + B\alpha_c)(T - T_0) \quad (14)$$

where $I(T)$ and $I(T_0)$ are the intensities at temperatures T and T_0 , respectively, and A and B are constants which are given by

$$A = \frac{\frac{2\rho_{s0}}{\Delta\rho_0} + \left(1 - \frac{2r_{c0}}{a_0} \right) \left(2 - \frac{2r_{c0}J_0(2\pi r_{c0}/ma_0)}{a_0J_1(2\pi r_{c0}/ma_0)} \right) - 2\rho_{s0}r_{c0}J_1\left(\frac{\pi}{m}\right)}{1 + \frac{a_0\rho_{s0}J_1(\pi/m)}{2\Delta\rho_0r_{c0}J_1(2\pi r_{c0}/ma_0)}} \quad (15)$$

$$B = \frac{\frac{\rho_{s0} - 3\rho_{c0}}{\Delta\rho_0} + \left(1 - \frac{2r_{c0}}{a_0} \right) \frac{2\pi r_{c0}J_0(2\pi r_{c0}/ma_0)}{ma_0J_1(2\pi r_{c0}/ma_0)} + \frac{4r_{c0}}{a_0} + \frac{\rho_{s0}}{2} (4r_{c0} - a_0)J_1\left(\frac{\pi}{m}\right)}{1 + \frac{a_0\rho_{s0}J_1(\pi/m)}{2\Delta\rho_0r_{c0}J_1(2\pi r_{c0}/ma_0)}} \quad (16)$$

where $\Delta\rho_0$ is the difference between the electron density of the helical core ρ_c and that of the side-chain region ρ_s .

Equation 14 indicates that the square root of the relative intensity is a linear function of tempera-

ture with a slope of $A\alpha_s + B\alpha_c$. If the slope is positive, the intensity increases with increasing temperature; and if negative, the intensity decreases. In the next section, we shall estimate A and B for the particular cases of PMLG and PBLG.

APPLICATION

The crystalline form of PMLG is hexagonal, containing one chain, with a unit cell edge of $a=11.95 \text{ \AA}$ and an axial repeat of $c=27 \text{ \AA}$, corresponding to an 18-residue 5-turn helix.³ Therefore, we assume that the total helix and the main-chain helix have a radius of $r_s=5.98 \text{ \AA}$ and $r_c=3.9 \text{ \AA}$, respectively. The latter corresponds approximately to the outside of the β -carbon.⁵ Thus the electron densities ρ_c and ρ_s are estimated as 1.58 and 1.29, respectively, and $\Delta\rho_0$ is 0.29. The value of m for the (100) reflection is $\sqrt{3}/2$ from eq 11. Substituting these values into eq 15 and 16, A and B are 3.3 and -4.3 , respectively. The square root of the relative intensity of the (100) reflection for PMLG is then given by

$$\left\{ \frac{I(T)}{I(T_0)} \right\}^{1/2} = 1 + (3.2\alpha_s - 4.3\alpha_c)(T - T_0) \quad (17)$$

Accordingly, eq 17 indicates that if $\alpha_s > 1.5\alpha_c$, the intensity increases with increasing temperature. The linear thermal expansion coefficient of the helical core α_c is thought to be the same order of magnitude as that of 1.5 \AA -meridional spacing, which is the axial translation per amino acid residue. The thermal expansion coefficient of the 1.5 \AA spacing is one-tenth as large as that of the lateral spacing.⁶ This completely satisfies $\alpha_s > 1.5\alpha_c$. Therefore, the square root of the intensity increases linearly with increasing temperature. This prediction is in satisfactory agreement with the results obtained for the (100) reflection for PMLG, as shown in Figure 1. The coaxial two phase model seems to be very applicable.

From the slope of the straight line shown in Figure 1, we can estimate the linear thermal expansion coefficient of the side-chain region by assuming that the thermal expansion coefficient of the helical core is the same as that of the 1.5 \AA -meridional spacing. Table I shows the estimated values of the linear thermal expansion coefficient of the side-chain region; α_{1s} and α_{2s} refer to the ones observed above and below the transition temperature, T_t , which is defined as the break point temperature in Figure 1, respectively.¹ Table I also includes experimental values obtained by dilatometric measurements, which are corrected to give the linear thermal expansion coefficient in the side-chain region.¹ Although the model is

Table I. Linear thermal expansion coefficient of the side-chain region for PMLG and PBLG

PMLG		
	$\alpha_{1s}(T > T_t^a),$ $^{\circ}\text{C}^{-1}$	$\alpha_{2s}(T < T_t^a),$ $^{\circ}\text{C}^{-1}$
Intensity of the (100) reflection	5.6×10^{-4}	2.3×10^{-4}
Specific volume	4.6×10^{-4b}	2.1×10^{-4b}
PBLG		
	$\alpha_{1s}(T > T_t^a),$ $^{\circ}\text{C}^{-1}$	$\alpha_{2s}(T < T_t^a),$ $^{\circ}\text{C}^{-1}$
Intensity of the ($1\bar{1}0$) reflection	6.5×10^{-4}	2.2×10^{-4}
Specific volume	4.2×10^{-4b}	2.0×10^{-4b}

^a T_t , transition temperature.

^b By K. Hikichi, A. Tsutsumi, S. Isozaki, and M. Kaneko.¹⁰

very approximate in nature, the agreement between values obtained by the two methods is close.

The unit cell of PBLG is triclinic, containing one chain, with cell dimensions of $a=b=15.25 \text{ \AA}$, $c=27 \text{ \AA}$ (fiber axis), $\alpha=\beta=84^{\circ}20'$, and $\gamma=122^{\circ}$, corresponding to an 18-residue 5-turn helix.⁴ This is a minor modification of the hexagonal lattice. Thus, we assume that PBLG is arranged in a hexagonal lattice, whose unit cell dimensions are $a=b=14.93 \text{ \AA}$ and $c=27 \text{ \AA}$. In this case the radius of the total helix r_s and of the main-chain helix r_c are 7.47 \AA and 3.9 \AA , respectively. The electron densities ρ_c and ρ_s are then estimated to be 1.58 and 1.32, and $\Delta\rho_0$ is 0.26. Using these values, we finally obtain the following equation for the temperature-dependence of the ($1\bar{1}0$) reflection intensity.

$$\left\{ \frac{I(T)}{I(T_0)} \right\}^{1/2} = 1 + (3.3\alpha_s - 4.3\alpha_c)(T - T_0) \quad (18)$$

As in the case of PMLG, eq 18 clearly indicates that the square root of the ($1\bar{1}0$) reflection intensity for PBLG increases linearly with increasing temperature. This agrees with the experimental results of the ($1\bar{1}0$) reflection for PBLG.¹ The linear thermal expansion coefficients of the side-chain region obtained by X-ray intensity and dilatometric measurements are shown in Table I.

We may estimate the temperature-dependence of other reflections by using α_s and α_c obtained above. For example, for the ($1\bar{1}0$) reflection of

PMLG, we expect $A\alpha_s + B\alpha_s = -4.0 \times 10^{-4}$ above the transition temperature T_t . However, the experimental value was from 0 to -9×10^{-4} , although smaller than 15.6×10^{-4} for the (100) reflection.¹ The reason for this disagreement is that the (1 $\bar{1}$ 0) reflection is related to a more detailed structure of the rod which we neglect in this model.

Similarly, for the (2 $\bar{1}$ 0) reflection of PBLG, we expect $A\alpha_s + B\alpha_s = -17 \times 10^{-4}$ above T_t . The experimental value was positive. This shows that the slope is opposite in sign between the theoretical and experimental values. The disagreement is caused by the same reason as that of the (1 $\bar{1}$ 0) reflection of PMLG.

The change in slope at T_t for PMLG and PBLG was interpreted in terms of the onset of the side-chain motions.¹ This also has been confirmed by many physical methods, including nuclear magnetic resonance, dynamic mechanical and dielectric measurements.^{5,7-9}

CONCLUSION

We proposed a structural model for solid esters of poly(glutamic acid) in the α -helical state. We designate a coaxial two phase model consisting of rigid helical core and soft side chain. An equation of lateral reflection intensity from the crystal composed of the coaxial rod was derived, enabling us to treat quantitatively temperature-dependence

of the intensity of the lateral reflection. The equation was successfully applied in analyzing the experimental results for PMLG and PBLG.

Acknowledgment. One member of our group (N. M) acknowledges his sincere appreciation to the Japan Association for the Scientific Promotion for the Grant of a Research Fellowship. This work was supported by a grant-in-aid from the Ministry of Education.

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