X-Ray Scattering of Synthetic Poly(α -amino acid)s in the Solid State. I. Transition of Poly(γ -benzyl L-glutamate) and Poly(γ -methyl L-glutamate)

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ABSTRACT: Lateral spacings between helices and corresponding X-ray diffraction intensities for poly(γ -benzyl L-glutamate) and poly(γ -methyl L-glutamate) in the solid state have been measured as a function of temperature from -100 to 100°C . The integrated intensity increased with increasing temperature and moreover showed breaks in the vicinity of room temperature, where breaks were also observed in the spacing-temperature relation. The temperature dependence of the intensity is approximately interpreted in terms of the same concept encountered in the small-angle X-ray scattering for crystalline polymers. The breaks observed are considered to be a transition analogous to the glass transition which occurs in a paracrystalline region and is related to side-chain motion. The results seem to suggest that the side chain does not assume any definite structure above the transition temperature.

KEY WORDS X-Ray / Spacing / Intensity / Poly(γ -benzyl L-glutamate) / Poly(γ -methyl L-glutamate) / Side-Chain Motion /

It has been well established that several synthetic poly(α -amino acid)s, such as poly(γ -benzyl L-glutamate) (PBLG) and poly(γ -methyl L-glutamate) (PMLG), have the α -helical molecular conformation in solutions and in the solid state.^{1,2} Structural investigations of oriented fibers of PBLG show that the polymer is paracrystalline, composed of approximately hexagonally packed α -helices with random displacement along the fiber axis, screw dislocations, and probably a random chain sense.^{3,4} PMLG molecules are reported to be packed into a hexagonal unit cell with the α -helical conformation.¹ No atomic positions of side chains of PBLG and PMLG have been reported.

Recently, interest has grown in the study of the conformations which the side chain adopts. Squire and Elliott^{5,6} have suggested that an ordered arrangement of the side chains is likely to occur in the liquid-crystalline phase of PBLG, and in the solid mixture of equal parts by weight of this polymer and its D isomer.

At the same time, molecular motions occurring in these synthetic poly(α -amino acid)s in the solid

state have been widely studied.⁷⁻¹⁴ Experimental results for PBLG and PMLG have shown that considerable side-chain motions are liberated near room temperature for these polymers.

McKinnon and Tobolsky^{10,11} studied creep modulus and specific volume as a function of temperature for PBLG. They showed that the solid film of PBLG contains both paracrystalline and mesomorphic regions. Further, the mesomorphic regions are noncrystalline region whose density is lower than that of the paracrystalline regions. They also stated that a glass transition occurs at 12°C , due to the onset of side-chain motion in the mesomorphic region. These results of studies on side-chain motion seem to indicate that the structures of the side chain is not definitely determined, in contrast with the results of the structural study by Squire and Elliott.

The purpose of the present work is to obtain a more detailed understanding of such studies. X-ray diffraction measurements for PBLG and PMLG in the solid state were performed to examine the lateral spacings between α -helices and the intensities of Bragg reflections from the

lateral planes as a function of temperature from -100 to 100°C . The results obtained were interpreted in terms of side-chain structure.

EXPERIMENTAL

PBLG and PMLG samples used in this work were obtained by polymerization from the respective *N*-carboxy anhydrides in dioxane and methylene chloride. The molecular weights of PBLG and PMLG, determined by the *N*-terminal titration, are about 93000 and 35000, respectively.

Samples for X-ray measurements were in the form of film. A film of PBLG was cast from concentrated chloroform solution on a glass plate. The film was repeatedly washed with methanol, and then dried at 60°C *in vacuo* for two days to remove residual solvents. The film was annealed at a temperature of 108°C for 8 hr. A film of PMLG was cast from the solution polymerized in methylene chloride. Annealing was carried out at a temperature of 120°C for 4 hr after drying in the same way as PBLG. In these films, the molecules are all believed to take the α -helical conformation.^{1,2}

X-ray measurements were performed with a Rigaku Denki diffractometer. The nickel-filtered $\text{Cu-K}\alpha$ radiation was detected by a scintillation counter with a pulse-height analyzer. A symmetrical-reflection (SR) technique,¹⁵ in which the normal of a film is maintained at an angle of $90^\circ - \theta$ with respect to the incident and diffracted beams, was employed to measure the X-ray intensity and the spacing for PMLG and the spacings for PBLG; 2θ is the Bragg angle.

Measurements of the intensity of PBLG were performed by a fixed time method and a symmetrical-transmission (ST) technique,¹⁵ in which the normal of a film is maintained at an angle of θ with respect to the incident and diffracted beams. Intensities were counted for 60 sec at intervals of 0.1° in Bragg angle in the central region of the peak and at intervals of 0.2° in the vicinity of the tails.

The temperature of samples was changed from -100 to 100°C . In the temperature range from -100°C to room temperature, the sample was cooled by a stream of gas evaporated from liquid nitrogen. The temperature was adjusted by controlling the boiling rate of liquid nitrogen. Above

room temperature, the sample was heated by blowing hot air. The temperature was measured by a copper—constantan thermocouple.

RESULTS AND DISCUSSION

Bamford, *et al.*,¹⁶ have suggested that the unit cell of PBLG is triclinic, containing one chain, with the cell dimensions of $a=b=15.25\text{ \AA}$, c (fiber axis) $=27.0\text{ \AA}$, $\alpha=\beta=84^\circ20'$, and $\gamma=122^\circ$. This may be regarded as a minor modification of the corresponding hexagonal cell. There is a great deal of evidence indicating some preference for the (110) planes to be parallel to the cast film of PBLG.^{1,11}

Since the X-ray diffraction scans obtained by the SR technique approximately correspond to photographs taken with the beam parallel to the plane of the film, the Bragg reflections observed at $2\theta \approx 7^\circ$ and 12° are indexed as (100) and (010), and (110), respectively. On the other hand, the Bragg reflection at $2\theta \approx 6^\circ30'$ observed by ST is indexed as (1 $\bar{1}$ 0), since this method approximately gives diffraction patterns taken with the beam normal to the film.

According to Bamford, *et al.*,¹⁷ the unit cell of PMLG is taken to be a hexagonal lattice with $a=b=11.95\text{ \AA}$ and c (fiber axis) $=27.0\text{ \AA}$, containing one chain. Therefore, the Bragg reflection at $2\theta \approx 10^\circ$ is indexed as (100) and (010).

In Figure 1, the spacings of the (100) and (110) reflections of PBLG are plotted against temperature. Both spacings were found to increase linearly with increasing temperature. It is of interest to note that the spacing—temperature

Figure 1. Spacings of the (100) and (110) Bragg reflection of PBLG as a function of temperature.

Figure 2. Spacing of the (100) Bragg reflection of PMLG as a function of temperature.

curve has a break in the vicinity of room temperature.

Likewise, the spacing of the (100) reflection of PMLG shows a linear temperature dependence, which has a break at about 0°C, as illustrated in Figure 2.

Breaks have been reported in the specific volume—temperature curve for PBLG and PMLG at about 16°C and -8°C, respectively.¹⁸ Such breaks have been considered as manifestations of the glass transition, which is due to the onset of side-chain motion. It is, therefore, reasonable to assume that the breaks observed in Figures 1 and 2 result from the same origin as those observed in the specific volume—temperature curve.

Since only the crystalline or paracrystalline phase is responsible for the Bragg reflection, the observed breaks are attributed to the crystalline or paracrystalline phase as well as to the mesomorphic noncrystalline phase if it exists. It is, therefore, obvious that the side-chain motion occurs in the paracrystalline region. This is contrary to the suggestion by McKinnon and Tobolsky that the side-chain motion occurs in the mesomorphic region.¹⁰

Integrated intensities for the (1 $\bar{1}$ 0) reflection of PBLG and for the (100) reflection of PMLG were measured as a function of temperature. It was found that the intensities for these reflections increase with increasing temperature.

A qualitative account for this result may be found in the differences between the temperature factors of atoms of the helical-core and of the side chains. It is reasonable to assume that the temperature factors of side-chain atoms and the

dependence on temperature of these factors are greater than those of the helical core, because of side-chain motions. Referring to the molecular packing and dimensions of the helical core and side chains,¹ the contributions from both to the molecular structure factors for these reflections are opposite in sign. The contribution from the side chains to the molecular structure factor has greater temperature factors. Therefore, the intensity will increase with increasing temperature.

A quantitative account along this line will require a detailed structure analysis at each temperature, which we do not intend to do here. Instead of doing such a justified but practically impossible procedure, we will take a different approach.

Such an increase in intensity reminds us of small-angle X-ray scattering (SAXS) studies on crystalline polymers, for which the intensity of SAXS increases with increasing temperature. This has been interpreted in terms of a "two phase model", which describes the sandwich structure of crystalline polymers consisting of two regions differing in electron density, *i.e.*, crystalline lamella and amorphous surface layer.

Bamford, *et al.*,¹ presented the radial section of the electron density projected on the basal plane for PMLG; this is shown in Figure 3.

Figure 3. Radial section of electron density projected on basal plane for PMLG.¹ The abscissa indicates the distance (r) from the axis of the α -helix. The dotted line indicates a model of the rectangular density profile for PBLG and PMLG.

This may be roughly approximated by a rectangular shape, as shown by the dotted line in Figure 3; this type of electron density distribution is widely used for SAXS studies of crystalline polymers.

Thus, we are tempted to apply the concept of a "two phase model" to the present case. It is reasonable to assume two regions, the helical core and the side-chain region, corresponding to the crystalline lamella and amorphous surface layer, because the unit cell contains only one α -helical chain.

According to the SAXS theory for crystalline polymers,¹⁹ the intensity is proportional to the product of the lattice factor and the square of the structure factor, the latter being related to the electron density difference between the two phases and their volume fractions. Since the lattice factor and the volume fraction are insensitive to temperature, and the electron density is proportional to the mass density, the temperature dependence of the intensity is written as

$$\sqrt{\frac{I(T)}{I(T_0)}} = 1 + \frac{\alpha_s \rho_s - \alpha_c \rho_c}{\Delta \rho} (T - T_0) \quad (1)$$

where ρ_s and ρ_c are the densities of the side-chain region and helical core at a reference temperature T_0 , $\Delta \rho$ is the difference between the two, and α_s and α_c are the thermal expansion coefficients of the side-chain region and helical core. Equation 1 indicates that the square root of the relative intensity is a linear function of temperature with a slope of $(\alpha_s \rho_s - \alpha_c \rho_c) / \Delta \rho$.

Figures 4 and 5 show the square root of the integrated intensity plotted against temperature

Figure 4. The relative change of the square root of the (110) Bragg reflection intensity of PBLG as a function of temperature.

Figure 5. The relative change of the square root of the (100) Bragg reflection intensity of PMLG as a function of temperature.

for the (110) reflection of PBLG and the (100) reflection of PMLG, respectively. The intensity was measured in units of the intensity at 19°C for the (110) reflection of PBLG, and at 16°C for the (100) reflection of PMLG. Straight lines with breaks in the vicinity of 19°C for the (110) reflection of PBLG and 0°C for the (100) reflection of PMLG were obtained, as seen in Figures 4 and 5. It is clear that these breaks are related to the ones observed in the spacing—temperature curve.

Because of the rigidity of the helical core, we expect the thermal expansion coefficient of the helical core, α_c , to be negligibly small at the temperatures studied. Therefore, the change in slope indicates that a sudden change in the thermal expansion coefficient of the side-chain region, α_s , occurs at these temperatures. This fact indicates that a transition analogous to the glass transition is taking place in the side-chain region.

On the basis of the results of dynamic mechanical and dielectric measurements for these polymers, Yamashita, *et al.*,¹³ and Tsutsumi, *et al.*,¹⁴ have suggested that the side-chain relaxation of these polymers occurring above the transition temperature has a close resemblance to the relaxation accompanying the glass transition. The present result supports this suggestion.

Further evidence for this is obtained from the value of the slope of the intensity—temperature curve. The densities of the helical core ρ_c and the side-chain region ρ_s were reported to be 1.58 and 1.17 for PBLG, and 1.58 and 1.12 for PMLG, respectively.¹³ The observed values of the slope

for the (110) reflection of PBLG are $20.6 \times 10^{-4}/^{\circ}\text{C}$ and $6.8 \times 10^{-4}/^{\circ}\text{C}$ above and below 19°C respectively. For the (100) reflection of PMLG, we obtained values $15.6 \times 10^{-4}/^{\circ}\text{C}$ and $6.0 \times 10^{-4}/^{\circ}\text{C}$ above and below 0°C respectively. Using these values, we can estimate the thermal expansion coefficient of the side-chain region α_s , assuming $\alpha_0=0$.

Table I shows the estimated values of the thermal expansion coefficient of the side-chain region; α_{1s} and α_{2s} refer to the ones above and below the transition temperature, respectively, and $\Delta\alpha_s$ to the difference between the two. Table I also includes experimental values obtained by dilatometric measurements, which were corrected to give the thermal expansion coefficient in the side-chain region, assuming only the existence of the crystalline or paracrystalline region. Taking account of the approximate nature of the analysis made here, we obtained a fairly close agreement between the values obtained by the X-ray intensity and the dilatometric measurements. In the case of the glass transition, $\Delta\alpha_s$ has been reported to be $4.8 \times 10^{-4}/^{\circ}\text{C}$.²⁰ The agreement in PBLG is probably accidental.

The results obtained here suggest that the solid films of these polymers consist of a homogeneous crystalline or paracrystalline region. The existence of a mesomorphic region is doubtful. It

would seem unlikely that the side chain assumes a definite structure above the transition temperature, because the side-chain motion occurs in the crystalline or paracrystalline region. This is in agreement with a conclusion by Flory and Leonard²¹ that the side chains of PBLG are disordered. It should be noticed that the transition observed for PBLG and PMLG is similar to a crystalline (paracrystalline) phase transition of the second order as well as to the glass transition.

CONCLUSIONS

The results of measurements of the lateral spacings between α -helices and the intensity of these Bragg reflections for PBLG and PMLG as a function of temperature clearly show that the solid films of these polymers manifest a transition analogous to the glass transition near 19°C and 0°C , respectively. The transition occurs in the paracrystalline region and is related to the side-chain motion. The existence of the mesomorphic region is doubtful. These results seem to suggest that the side chain does not assume any definite structure above the transition temperature.

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Table I. Thermal expansion coefficients of the side-chain region for PBLG and PMLG

	$\alpha_{1s}(T > T_t),$ $^{\circ}\text{C}^{-1}$	$\alpha_{2s}(T < T_t),$ $^{\circ}\text{C}^{-1}$	$\Delta\alpha_s$ $^{\circ}\text{C}^{-1}$
PBLG			
Intensity of the (110) reflection	7.2×10^{-4}	2.4×10^{-4}	4.8×10^{-4}
Specific volume	8.2×10^{-4} ^a	2.8×10^{-4} ^a	5.4×10^{-4} ^a
	6.8×10^{-4} ^b	3.2×10^{-4} ^b	3.6×10^{-4} ^b
PMLG			
	$\alpha_{1s}(T > T_t),$ $^{\circ}\text{C}^{-1}$	$\alpha_{2s}(T < T_t),$ $^{\circ}\text{C}^{-1}$	$\Delta\alpha_s$ $^{\circ}\text{C}^{-1}$
Intensity of the (100) reflection	6.3×10^{-4}	2.4×10^{-4}	3.9×10^{-4}
Specific volume	8.9×10^{-4} ^a	4.4×10^{-4} ^a	4.5×10^{-4} ^a

^a By A. Tsutsumi, S. Isozaki, K. Hikichi, and M. Kaneko.¹⁸

^b By A. J. McKinnon and A. V. Tobolsky.¹⁰

^c T_t , Transition temperature.

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