

X-Ray Scattering of Synthetic Poly(α -amino acid)s in the Solid State. II. Phase Transition of DL Mixtures of Poly(γ -benzyl glutamate)s

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ABSTRACT: Spacings and intensities of Bragg reflections for mixtures of poly(γ -benzyl-L-glutamate) (PBLG) and poly(γ -benzyl-D-glutamate) (PBDG) were measured as a function of temperature from -70 to 110°C . Mixtures of L and D molecules were found to show a transition due to side-chain motion in the vicinity of 25°C and a first-order phase transition in the vicinity of 80°C . From the intensity of the $10.6\text{-}\text{\AA}$ reflection which is caused by regular stacks of benzyl groups, the degree of stacking S was estimated at each temperature. The temperature dependence of S thus obtained was compared with that calculated on the basis of a theory proposed by Hoffman. The phase transition at 80°C can be interpreted in terms of the order—disorder transition, which is caused by the transition of benzyl groups from the stacked state to the unstacked state.

KEY WORDS X-Ray / Spacing / Intensity / Poly(γ -benzyl glutamate)s / Stacks / Order—Disorder Transition /

Recently, interest has been growing in the structure of mixtures of poly(γ -benzyl-L-glutamate) (PBLG) and poly(γ -benzyl-D-glutamate) (PBDG).¹⁻⁵ X-ray diffraction studies on the dry fibers of this mixture have shown that backbone structure of this polypeptide is an α -helix with 43 residues in 12 turns, although a strong layer line of 10.6 \AA and other weaker lines not expected from the α -helix occur in the diffraction patterns. Squire and Elliott have presented a model to interpret these layer line.⁵ The model involves regular stacks of benzyl groups from about five side chains. The stacks are formed by interaction between adjacent molecules of opposite screw sense; every second benzyl group in the stacks originates from the same molecule.

Uematsu, *et al.*,⁶⁻⁹ have reported that the mixtures exhibit a reversible first-order solid—solid phase transition at a temperature of about 90°C . They suggested that the phase transition is caused by the breakdown of regular stacks of benzyl groups, as evidenced by the disappearance of the $10.9\text{-}\text{\AA}$ reflection (corresponding to 10.6 \AA) above the transition temperature.

The present authors¹⁰ carried out dielectric

measurements for these mixtures to investigate the behavior of the phase transition, and showed that the results obtained are consistent with a previous idea proposed by Uematsu, *et al.*

To obtain further information about the transition, X-ray diffraction studies were carried out for oriented specimens of the mixtures as a function of temperature.

EXPERIMENTAL

PBLG and PBDG samples used in this work were prepared by polymerization of *N*-carboxy anhydrides in dioxane. The molecular weights determined from their viscosities measured in *N,N*-dimethylformamide are approximately equal to one another, being 1.58×10^5 and 1.62×10^5 for PBLG and PBDG, respectively.

Two oriented films were examined in this work. These were a mixture of equal parts by weight of PBLG and PBDG (hereafter abbreviated as PBLG+PBDG (1:1)) and a mixture of the L-to-D ratio 3:1 (PBLG+PBDG (3:1)). The oriented films were prepared by using one glass plate to stroke the cocentrated chloroform solution in one direction until it

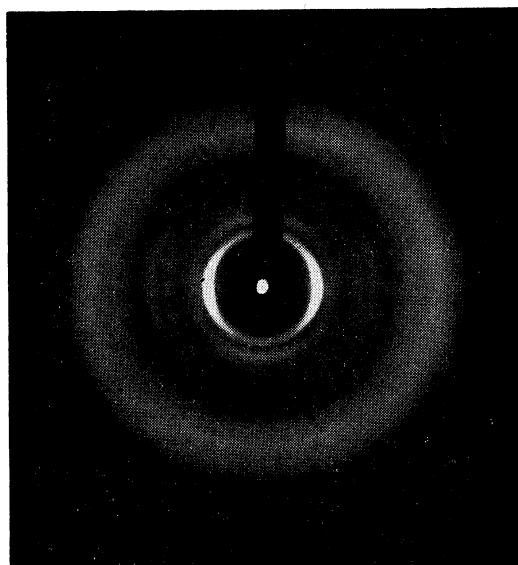
dried on a second glass plate. The films were heated *in vacuo* at 120°C to remove the chloroform.

X-ray photographs were obtained with a flat camera of specimen-to-film distance 50 mm using Cu-K α radiation, the incident beam being perpendicular to the film plane. X-ray measurements were performed with a Rigaku Denki diffractometer using a scintillation counter with a pulse height analyzer and Ni-filtered Cu-K α radiation. The X-ray line profiles were obtained in the symmetrical-transmission technique,¹¹ in which the plane of the film was maintained at angle $(90-\theta)$ with respect to both the incident and diffracted beams, θ being the Bragg angle. In order to measure diffraction profiles on the meridian and equator the oriented films were set in such a way that the directions of orientation were perpendicular and parallel to the rotational axis of the diffractometer, respectively. Only the radiation scattered in the direction of $2\theta=8^{\circ}20'$ was measured, using a point-to-point technique and a fixed time method to ensure accuracy in intensity estimation. Intensities were then counted for 100 sec at intervals of 0.05° in the Bragg angle in the peak and at intervals of 0.1° in the vicinity of the tails. The background was established by drawing a smooth curve from tail to tail. The temperature was changed from -70 to 110°C and measured by a copper-constantan thermocouple.

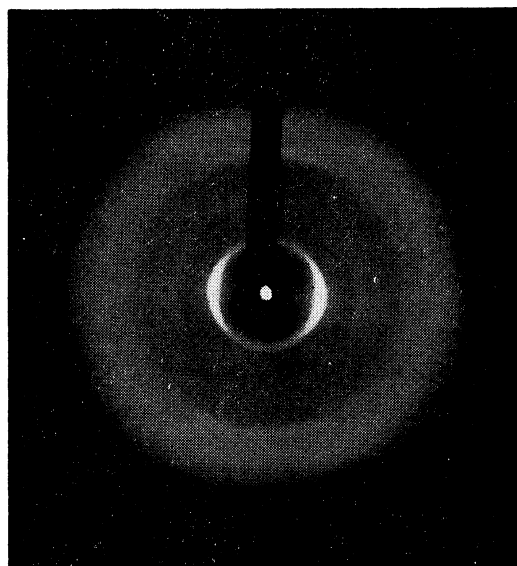
RESULTS AND DISCUSSION

Figures 1a and b show flat-plate X-ray photographs of PBLG+PBDG (1:1) at room temperature and 90°C , respectively. The 10.6-Å reflection on the meridian, which is reported to be related to the regular side-chain structure, clearly disappears above the phase transition.

Figure 2 shows the diffraction profiles for PBLG+PBDG (1:1) scanned in the equatorial and meridional directions. Referring to the result by Squire and Elliott,⁵ the reflections on the equator at $2\theta=6^{\circ}45'$ can be indexed as (110) and (020), which correspond to lateral spacings between α -helices. The (110) reflection occurs also on the meridian, presumably due to an imperfect orientation of the sample. The reflection on the meridian at $2\theta=8^{\circ}20'$ corresponds



(a)



(b)

Figure 1. Flat-plate X-ray photographs of PBLG+PBDG (1:1) at room temperature (a) and 90°C (b).

to a spacing of 10.6 Å.

Figure 3 shows a plot of the spacing of the (110) reflection as a function of temperature. The spacing linearly increases with increasing temperature from -70 to 25°C , where an abrupt change in slope occurs. The linear thermal ex-

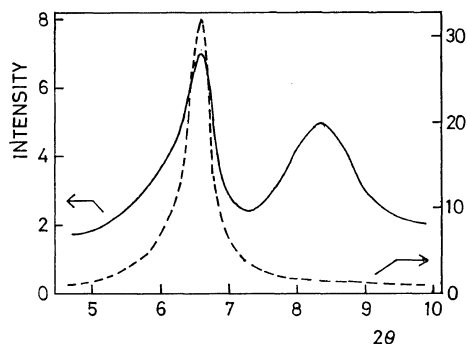


Figure 2. Diffraction profiles in a range from $2\theta=5$ to 10° for PBLG+PBDG (1:1), θ being the Bragg angle: —, meridional; ----, equatorial.

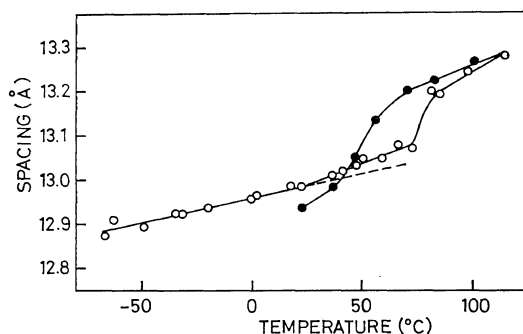


Figure 3. Spacing of the (110) Bragg reflection of PBLG+PBDG (1:1) as a function of temperature: ○, heating; ●, cooling.

pansion coefficient in the $\langle 110 \rangle$ direction is $0.9 \times 10^{-4}/^\circ\text{C}$ and $1.5 \times 10^{-4}/^\circ\text{C}$ just below and above 25°C , respectively. Furthermore, it is of interest to note further heating gives rise to a small but definite sudden change in the spacing (about 0.7%) in the vicinity of 80°C .

In a previous paper, we reported that such a discontinuity in slope was also observed for pure PBLG and poly(γ -methyl-L-glutamate) (PMLG).¹² It is, therefore, evident that the discontinuity in slope at 25°C is due to the same origin as those observed for PBLG and PMLG, in which it is attributed to the onset of side-chain motion.

The sudden increase in the spacing observed in the vicinity of 80°C indicates that there occurs a first-order phase transition in the mixture of PBLG and PBDG. Uematsu, *et al.*,⁶ reported results of dilatometric measurements

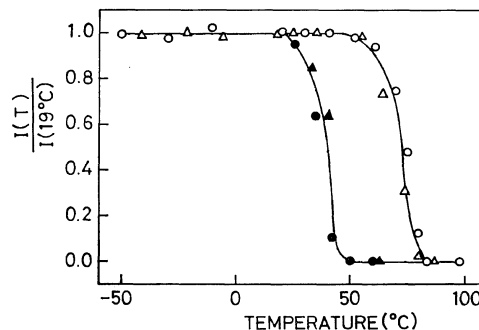


Figure 4. The $10.6\text{-}\text{\AA}$ reflection intensity as a function of temperature for PBLG+PBDG (1:1) and PBLG+PBDG (3:1): ○, PBLG+PBDG (1:1), heating; ●, PBLG+PBDG (1:1), cooling; △, PBLG+PBDG (3:1), heating; ▲, PBLG+PBDG (3:1), cooling.

for the racemic PBG. From their data the volume change at the transition temperature T_c is estimated to be about 1.2%. We also conducted dilatometric measurements and confirmed a 1.2% volume change at T_c .¹³ This indicates that the volume change is caused by an increase in lateral distance between α -helices associated with the transition.

Figure 4 shows the intensity of the $10.6\text{-}\text{\AA}$ reflection vs. temperature plots for PBLG+PBDG (1:1) and PBLG+PBDG (3:1), the intensity being measured in units of the one observed at 19°C . The temperature dependence of the intensity is almost the same for both samples over the entire temperature range studied. The intensity begins to decrease at a temperature of 50°C and vanishes completely at the transition temperature T_c . As the samples were cooled, the $10.6\text{-}\text{\AA}$ reflection appears abruptly at a temperature of 50°C ; a hysteresis behavior is observed. It is evident that the disappearance of the $10.6\text{-}\text{\AA}$ reflection in the vicinity of 80°C results from the same origin as the jump of the spacing of the (110) reflection.

According to a model presented by Squire and Elliott,⁵ for an L molecule only 31 benzyl groups out of the total of 43 in the repeat length interact with those of six neighboring D molecules to give stacks. Since in the case of the racemic PBG an L molecule is surrounded on the average by four neighboring D molecules and two Ls, 21 benzyl groups out of 43 are

anticipated to form the stacks. At no temperature are the stacks completely produced. We shall introduce the degree of stacking S , defined by the ratio of the number of stacked benzyl groups to the maximum number of benzyl groups which is possible to stack.¹⁰ Thus, using the degree of stacking S , the average structure factor F may be written as

$$F = SF_s + (1-S)F_{us} \quad (1)$$

where F_s is the structure factor when benzyl groups are all in the stacked state and F_{us} is the one for all in the unstacked state.

Since the 10.6-Å reflection does not appear for either pure PBLG or PBDG, it is reasonable to assume that the structure factor F_{us} does not contribute to this reflection at all. Consequently, the intensity of the 10.6-Å reflection is proportional to the square of the degree of stacking S . Since the intensity levels off at low temperatures, we assume that all the benzyl groups which can stack are in the stacked state at low temperatures; $S=1$. Thus, we estimated S as the square root of the intensity measured in units of the one at the lowest temperature. Figure 5 shows the degree of stacking S against temperature. The data obtained from dielectric measurements for PBLG+PBDG (1:1) are also shown for comparison. Taking account of the assumption used to estimate S , both results are in a qualitative agreement.

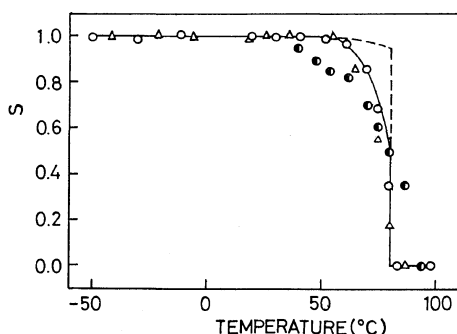


Figure 5. The degree of stacking S as a function of temperature for PBLG+PBDG (1:1) and PBLG+PBDG (3:1): ○, PBLG+PBDG (1:1); △, PBLG+PBDG (3:1). Dotted line shows a theoretical curve with $\Omega=60$. ● shows data of PBLG+PBDG (1:1) from dielectric measurements.¹⁰

If it is possible to examine theoretically the variation of S with temperature, one may obtain a more detailed understanding about the phase transition. Hoffman¹⁴ proposed a theory for a rotational transition of n -paraffin in the solid state using the Bragg Williams approximation. We applied a treatment similar to his theory to the present case, where the degree of stacking S is substituted for his "long-range rotational order."

Since the degree of stacking S is an order parameter, S will be written as

$$S = \frac{1}{1 + \Omega \exp(-ST_c/T)} \quad (2)$$

where Ω is the degeneracy of the unstacked state. T_c is defined as $T_c = V_0/R$, where V_0 is the energy required to change the state of a benzyl group from the stacked state to the unstacked one when $S=1$, and R the gas constant. S varies from 1 to $1/(1+\Omega)$ with changing temperature. If Ω is greater than e^2 , S becomes triple-valued over a certain range of temperature and this suggests that a first-order transition occurs. The dotted line in Figure 5 is a theoretical curve calculated from eq 2 with $\Omega=60$, which explains the experimental data most satisfactorily.

On the other hand, we can estimate Ω from thermodynamic data. If we know the heat of the transition ΔH_c , the entropy of transition ΔS_c is given by

$$\Delta S_c = \frac{\Delta H_c}{T_c} \quad (3)$$

ΔS_c must be the sum of the configurational entropy $R \ln \Omega$ and the entropy of expansion $\Delta S_{(\text{expansion})}$.¹⁴

$$\Delta S_c = R \ln \Omega + \Delta S_{(\text{expansion})} \quad (4)$$

$\Delta S_{(\text{expansion})}$ is given by

$$\Delta S_{(\text{expansion})} = 2.34 \times 10^{-8} \Delta V \cdot \alpha / \beta \quad (5)$$

where α is the cubical coefficient of expansion in deg^{-1} , β the cubical compressibility in $\text{cm}^2 \text{dyn}^{-2}$, and ΔV the change in molar volume at T_c in cm^3/mol .

For mixtures of PBLG and PBDG, α , β , and ΔV are 4.0×10^{-4} , 7.5×10^{-11} , and 1.8, respectively. β was estimated from the storage tensile

modulus of PBLG under the assumption that the Poisson ratio is 0.4. With these data $\Delta S_{(\text{expansion})} = 0.2$ eu. Using $T_c = 353\text{--}363^\circ\text{K}$ and $\Delta H_c = 3000\text{--}4000$ cal/mol reported by Masuko, *et al.*,⁹ we get $\Delta S_c = 8.2\text{--}11.3$ eu., *i.e.*, $\Omega = 50\text{--}130$. The agreement with the results from X-ray measurements is not so bad.

CONCLUSION

The temperature dependence of the spacing of the (110) reflection for mixtures of PBLG and PBDG clearly shows a first-order phase transition at a temperature of about 80°C . This indicates that the transition is accompanied by an increase in distance between the α -helices. It was found that the 10.6-\AA meridional reflection which is caused by regular stacks of benzyl groups vanishes at the transition temperature. The degree of stacking S was obtained from the intensity of the 10.6-\AA reflection. Hoffman's theory was reasonably applied to the temperature dependence of S . The degeneracy Ω of the unstacked state was found to be much larger than e^2 . All of the results obtained can be interpreted in terms of the order-disorder transition which is caused by the transformation of benzyl groups from the stacked state to the unstacked state.

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