

Molecular Motion in Poly(amino acid) VIII. β_2 -Form Crystals of Poly(D-glutamic acid)

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ABSTRACT: The β_2 -form crystal of poly(D-glutamic acid) has a strong interaction between the sheets, in which chains are bound side by side by intermolecular hydrogen bonds. In this work, the molecular motion of poly(D-glutamic acid) in the fully annealed β_2 -form crystal was studied. The linear thermal expansion coefficient for the intermolecular periodicity, 10^{-5} ($^{\circ}\text{C}^{-1}$), was similar to that for the intersheet periodicity below 200°C , indicating that there is only ordinary thermal lattice vibration in the β_2 -form crystal. This finding differs from that for the β -form crystal having no interaction between the sheets. Above 200°C , the side chains began to move as a result of weakening of the strong interaction between the sheets, leading to disordering and degradation of the β_2 -form crystal. In the amorphous region, two segmental micro-Brownian motions occurred at 120°C and above 140°C . The former was attributed to free amorphous chains which have no hydrogen bonding between the carboxyl groups of the side chains, and the latter to the weakening of hydrogen bonds.

KEY WORDS Poly(D-glutamic acid) / β_2 -Form Crystal / Amorphous Region / Molecular Motion / Hydrogen Bond / Side Chain /

Poly(D-glutamic acid), PGA, takes on two types of β -form crystal, *i.e.*, β_1 -form and β_2 -form, in which chains form a sheet by intermolecular hydrogen bonds as in the case of silk fiber,¹ poly(γ -methyl L-glutamate),² PMLG, and poly(L-alanine),² PLA. Keith *et al.*^{3,4} and Itoh *et al.*⁵ prepared β_2 -form crystal powder by direct precipitation from an aqueous solution of the sodium salt of PGA at low pH. Itoh *et al.*⁵ assigned the Miller indices for the X-ray reflections from the β_2 -form crystal, assuming the monoclinic unit cell with $a=9.75$ Å (direction of hydrogen bond), $b=6.84$ Å (fiber axis), $c=8.06$ Å (direction of the side chain) and $\beta=104^{\circ}$. They also found IR absorption bands differing from those for the ordinary β -form crystals in silk fiber, PMDG and PLA and attributed the difference to the strong interaction between the hydrogen bonded peptide groups of the side chains in an adjacent sheet. Takahashi *et al.*^{6,7} prepared a well oriented fiber containing only the β_2 -form crystal by spinning at a draw ratio of 2.7 from a concentrated aqueous solution of PGA containing 43% H_3PO_4 coagulant. They provided the orthorhombic unit cell with $a=$

9.26 Å (interchain periodicity in one sheet), $b=7.73$ Å (intersheet periodicity) and $c=6.90$ Å (fiber axis).

In our preceding papers on unannealed silk fiber^{8,9} and PLA,¹⁰ the temperature dependence of the spacings corresponding to the interchain and intersheet periodicities was measured and it was found that the slopes of those spacings against temperature increased above about 160°C . It was then proposed that the main chain motion occurs in the crystal as a result of weakening of intermolecular hydrogen bonds. For fully annealed PMDG¹¹ and silk fiber,¹² we clarified that the chains are packed very stably and densely in the sheet owing to strong intermolecular hydrogen bonds and that slippage of the sheets rather than the motion of the chains themselves takes place above about 200°C .

In this paper, a fully annealed β_2 -form crystal of PGA was used to study the molecular motion in the β -form crystal for poly(amino acid) having strong interaction between the hydrogen bonded peptide groups in one sheet and the side chain in an adjacent sheet. The spacings corresponding to the interchain

and intersheet periodicities were measured, and the interaction between the sheets were investigated as a function of temperature. The molecular motion in the amorphous region was discussed on the basis of hydrogen bonds between the side chains.

EXPERIMENTAL

Fine chips of PMDG were placed into a 75 wt% methanol aqueous solution containing 1.8 wt% sodium hydroxide, and saponified for 3 h at 20°C under stirring.⁶ The resulting sodium salt of PMDG was filtrated, washed by methanol and dried *in vacuo*. An aqueous solution of this salt was spun into a 43 wt% H₃PO₄ aqueous solution to obtain a PGA fiber. This undrawn PGA fiber was drawn to two times the original length, washed in water for one day, and then steam drawn further 2.5 times. The fibers so obtained were kept in a 4N HCl aqueous solution at 95°C for 5 days, annealed by heating to 200°C at a heating rate of 0.5°C min⁻¹, and held at this temperature for 0.5 h *in vacuo*.

X-Ray measurements were performed on a Rigaku Geigerflex 2028 diffractometer equipped with a scintillation counter and a pulse height analyzer, using CuK α radiation. The temperature was maintained within $\pm 0.5^\circ\text{C}$ under N₂ gas flow. X-Ray photographs were taken by a Rigaku Laue camera.

Infrared spectra were measured by a JASCO A-302 infrared spectrophotometer equipped with a heating device. The spectra for deuterated PGA were obtained by the KBr-tablet method in a dry gas atmosphere. The deuteration was performed in a sealed glass tube at 100°C for 100 h under D₂O vapor.

The volumetric thermal expansion was measured by a pyrex dilatometer at a heating rate of 2°C min⁻¹.

Thermal mechanical properties were measured on a Rigaku thermo-mechanical analyzer, TMA, at a heating rate of 10°C min⁻¹ in a N₂ gas atmosphere.

DSC thermograms were recorded on a Rigaku differential scanning calorimeter in a N₂ gas atmosphere.

The temperature dependence of the dynamic mechanical loss tangent, $\tan \delta$, was measured by a Rheovibron DDV-IIC dynamic viscoelastometer at a heating rate of 2°C min⁻¹.

Weight loss was measured on a Rigaku thermo-

gravity analyzer, TGA, at a heating rate of 10°C min⁻¹ under N₂ gas flow.

RESULTS AND DISCUSSION

The Laue photographs obtained were similar to that of the well oriented fiber containing the β_2 -form crystal prepared by Takahashi *et al.*⁷ The infrared spectrum showed absorption bands for amide I, II, III, and V at 1600, 1550, 1290, and 630 cm⁻¹, respectively, in agreement with Itoh *et al.*'s results for the β_2 -form crystal of PGA.⁵ Therefore, the PGA samples used were considered to consist only of the β_2 -form crystal.

Figure 1 illustrates the schematic model of Keith^{3,4} for the molecular conformation in the β_2 -form crystal of PGA. We can see two types of hydrogen bond; *i.e.*, (1) intermolecular hydrogen bonds and (2) interside chain hydrogen bonds between PGA molecules aligned antiparallel.

Figure 2 shows the temperature dependence of the spacings corresponding to the interchain periodicity in one sheet, d_{200} , and to the intersheet periodicity, d_{010} . These periodicities increase linearly up to 200°C, at which the slopes against the temperature change abruptly and become steeper. The linear thermal expansion coefficient, α , below 200°C was calculated from the plots in Figure 2, and is shown in Table I, where the results for the β -form crystal of PMDG¹¹ and silk fiber¹² are included. α_{200} corresponding to $\alpha_{\text{interchain}}$ and α_{010} corresponding to $\alpha_{\text{intersheet}}$ are of the same order of magnitude as $\alpha_{\text{interchain}}$ for PMDG and silk fiber. These results may support the suggestion of Itoh *et al.*⁵ that in the β_2 -form crystal there is a strong hydrogen bonding interaction between the hydrogen bonded peptide groups in one sheet and the carboxyl groups of the side chains in an adjacent sheet. The magnitude of the thermal expansion coefficients suggests that in the β_2 -form crystal there is only ordinary thermal lattice vibration in the stable crystal. The fact that $\alpha_{\text{interchain}}$ for PGA is larger than those for silk fiber and PMDG may indicate that the chain packing in the former is looser than in the latter, both along the hydrogen bonding direction. The increase in $\alpha_{\text{intersheet}}$ and $\alpha_{\text{interchain}}$ above 200°C may arise from the onset of an active motion or a chemical degradation. These speculations may be justified by the results from IR (Figures 4 and 6) and TGA (Figure 5) measurements.

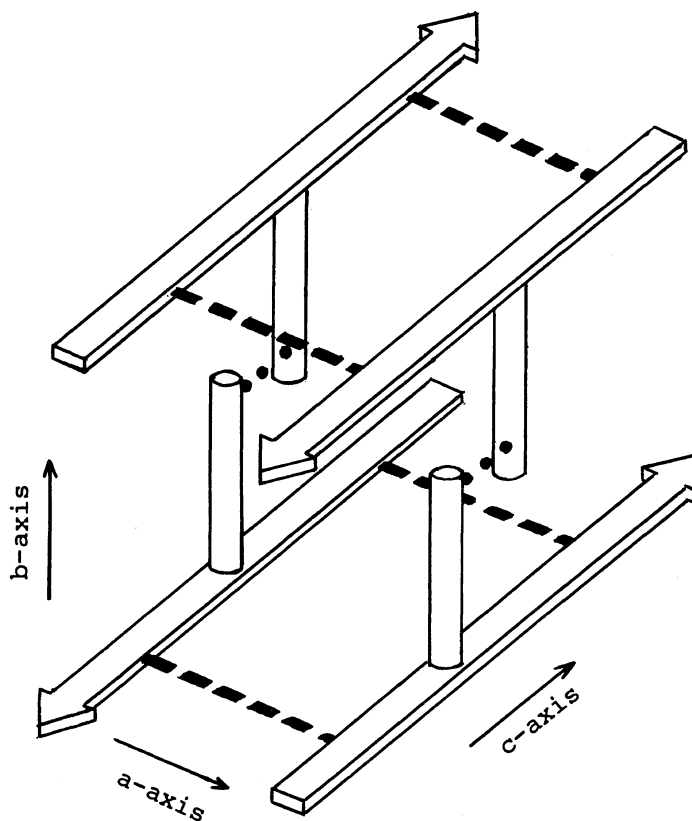


Figure 1. Schematic model of the β_2 -form crystal for PGA: \leftarrow , main chain; \square , side chain; \blacksquare , intermolecular hydrogen bonding; \bullet , interside chain hydrogen bonding.

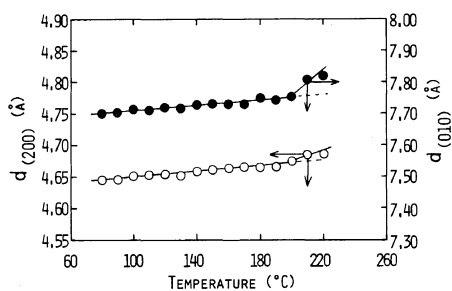


Figure 2. Temperature dependence of the spacings of 200 and 010 reflections for PGA.

Table I. Linear thermal expansion coefficients in the directions of interchain and intersheet periodicities for PGA

	$\alpha_{\text{Interchain}}$ $^{\circ}\text{C}^{-1}$	$\alpha_{\text{Intersheet}}$ $^{\circ}\text{C}^{-1}$
Silk fiber	2.0×10^{-5}	7.4×10^{-5}
Poly(γ -methyl D-glutamate)	2.1×10^{-5}	5.3×10^{-4}
Poly(D-glutamic acid)	5.4×10^{-5}	6.0×10^{-5}

Figure 3 shows the temperature dependence of X-ray diffraction intensities (I_{200} and I_{010}) for the 200 and 010 reflections. I_{200} and I_{010} increased linearly up to 200°C. Above 200°C they decrease abruptly and the binary crystalline peaks disappear at 230°C. The increase in I_{200} and I_{010} is caused by an increase

in the difference between the electron densities of the sheet and intersheet due to thermal expansion, not by an increase in the crystal size and order, because the sample was fully annealed. The disappearance of the crystalline peaks indicates that the lattice in the β_2 -form crystal becomes disordered

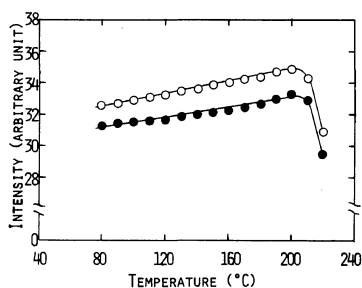


Figure 3. Temperature dependence of X-ray diffraction intensity for PGA: —○—, I_{200} ; —●—, I_{010} .

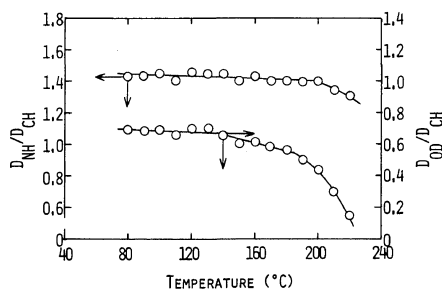


Figure 4. Temperature dependence of D_{NH}/D_{CH} and D_{OD}/D_{CH} for PGA.

just above 200°C. On the other hand, the sheet in the β -form crystal of silk fiber is very stable near this temperature.¹² This stability of silk suggests that the sheet in poly(amino acid)s having short side chains is stable.

Figure 4 shows the temperature dependence of the optical densities of the hydrogen bonded NH and OD stretchings normalized to the optical density of the CH stretching in the IR absorption bands of the deuterated sample (D_{NH}/D_{CH} and D_{OD}/D_{CH}). D_{OD}/D_{CH} starts to decrease gradually from 140°C, while D_{NH}/D_{CH} remains constant up to 200°C. Since the hydrogen atoms of the amorphous region of PGA may be easily deuterated, most of the observed undeuterated NH groups are considered to exist in the crystalline region. Thus, the result for D_{NH}/D_{CH} indicates that the intermolecular hydrogen bonds in the sheet are very stable up to 200°C, supporting the consideration on the interchain periodicity shown in Figure 2. The side chains in the β_2 -form crystal are also very stable up to 200°C as can be seen from Figure 2, while the result for D_{OD}/D_{CH} indicates that the hydrogen bonding force between the carboxyl groups of the side chains begins to weaken at 140°C. The hydrogen

atoms of the carboxyl groups in the amorphous and crystalline regions all can be deuterated under the condition of this work, according to Suzuki *et al.*¹³ Thus, our finding indicates the onset of the motion of hydrogen bonded side chains in the amorphous region. The fact that above 200°C D_{NH}/D_{CH} starts to decrease gradually implies that the weakening of intermolecular hydrogen bonding force occurs in the crystal. In the case of silk, the hydrogen bond does not weaken up to 240°C,¹² probably since the kinetic energy of the short side chain such as the methyl group is smaller than that of the intermolecular hydrogen bonding. For PGA, we may consider that as soon as the hydrogen bonds between the carboxyl groups of the side chains are weakened, the long side chain starts to move, since D_{OD}/D_{CH} decreases further. Therefore, the intermolecular hydrogen bonds in the sheet may be weakened by the kinetic energy of long side chains.

If an acid anhydride is formed from adjacent two carboxyl groups by dehydration, part of the produced water molecules reacts to break the peptide groups of the main chain:



Hence, the intermolecular hydrogen bonds are weakened. The production of water molecules accompanying the formation of an acid anhydride may be verified by the onset of weight loss above 205°C (Figure 5) and also by the appearance of IR absorption bands at 1790, 1740, and 1060 cm^{-1} which can be assigned to the acid anhydride in the heat-treated sample at 230°C (see Figure 6). In this way, the increase in $\alpha_{\text{intersheet}}$ and $\alpha_{\text{interchain}}$ and the decrease in I_{200} and I_{010} can be explained reasonably.

Figure 7 shows the temperature dependence of the volumetric thermal expansion for PGA. We can see a break at about 125°C and a more marked expansion above 145°C. Since no molecular motion occurs in the crystal up to 200°C, the breaking point corresponds to a glass transition point, T_g , *i.e.*, the onset of segmental micro-Brownian motion in the amorphous region. Two glass transitions take place for the β -form PMDG,¹⁴⁻¹⁶ one at room temperature and another at about 140°C, corresponding to the long side chains and the main chains, respectively. The chemical structure of PMDG, especially the side chain length, is similar to

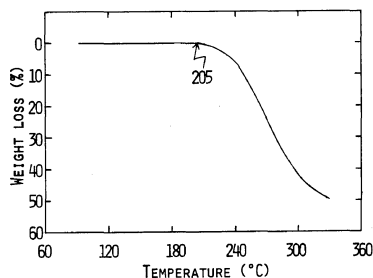


Figure 5. TGA thermogram for PGA.

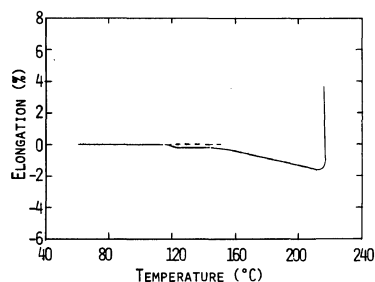


Figure 8. TMA thermogram for PGA.

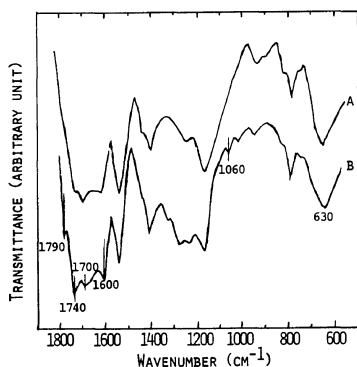


Figure 6. Infrared spectra for PGA: A, untreated; B, heat-treated at 230°C.

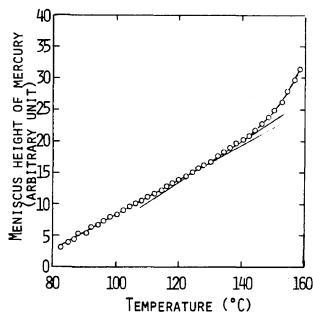


Figure 7. Temperature dependence of the volume thermal expansion for PGA.

that of PGA. Thus, the glass transition in PGA at 125°C may be attributed to the amorphous main chains. Furthermore, the fact that the hydrogen bond between the carboxyl groups of the side chains of the amorphous chains does not weaken up to about 140°C strongly suggests that the glass transition at 125°C is associated with free amorphous chains not hydrogen bonded. The above-mentioned

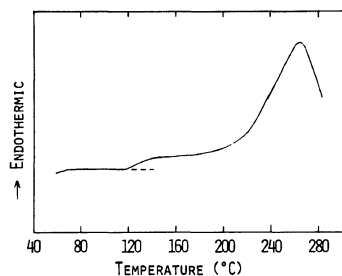


Figure 9. DSC thermogram for PGA.

expansion above 145°C may be attributed to the weakening of hydrogen bonds between the side chains.

A drawn and then fully annealed PGA sample may shrink at about T_g owing to the entropic force of the drawn amorphous chains. To see the shrinkage of a drawn PGA sample, TMA was measured under a small load and the curve shown in Figure 8 was obtained. The shrinkage occurred at two stages, one at about 120°C and another at 140°C. The first stage agrees with the dilatometric breaking point shown in Figure 7, indicating the unit of segmental micro-Brownian motion of the free amorphous chains. The second stage begins at a lower temperature than 200°C, at which the molecular motion in the β_2 -form crystal starts to occur, indicating that another type of segmental motion of hydrogen bonded chains occurs as a result of the weakening of hydrogen bonds. The continuous shrinkage above 200°C is interpreted by the entropic force arising from the motion of disordered main chains in the β_2 -form crystal. The TMA curve shows an abrupt elongation and break of the sample at 220°C due to degradation.

Figure 9 shows a DSC thermogram for PGA.

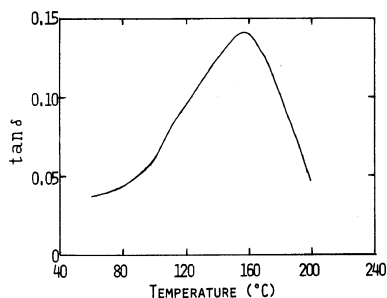


Figure 10. Temperature dependence of $\tan \delta$ for PGA.

There is a jump toward the endothermic side at about 120°C. This result again supports the conclusion that the break in dilatometry and the first stage of shrinkage on TMA at about 120°C are caused by segmental micro-Brownian motion of nonhydrogen bonded free amorphous chains. The gradual endothermic change above 140°C indicates weakening of the hydrogen bonds between the carboxyl groups of the side chains in the amorphous region, and the abrupt endothermic change above 200°C is due to dehydration of the side chain and successive degradation of the main chain.

From the results presented above, we expect that two types of segmental micro-Brownian motion should manifest themselves in the temperature dependence of $\tan \delta$. We measured $\tan \delta$ as a function of temperature at 3.5 Hz, and obtained the results shown in Figure 10. The curve is unsymmetric and has a moderate slope on the low temperature side of the maximum. We consider that the curve consists of two overlapped absorptions, *i.e.*, a major absorption at a higher temperature and a minor one at a lower temperature. Since, in general, dynamic viscoelastic properties measured at a low frequency may not be greatly different from static ones, the $\tan \delta$ curve in Figure 10 may be compared with the results from dilatometry, TMA and DSC. We conclude that the main absorption at about 155°C is due to the segmental micro-Brownian motion resulting from the weakening

of hydrogen bonds between the carboxyl groups of the side chains in the amorphous region. The shoulder of the $\tan \delta$ curve at about 120°C may reflect the glass transition due to the segmental micro-Brownian motion of the free amorphous chain which has no interside chain hydrogen bonding.

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