Viscoelastic Properties of Poly(γ -benzyl glutamate)

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ABSTRACT: The effect of solvents on the structure and properties of cast films of poly(γ -benzyl glutamate) (PBLG) were studied. From X-ray diffraction measurements, it was found that films cast from dichloroethane, chloroform, and dichloromethane (called film A) had a well-ordered structure consisting of helices packed on a regular hexagonal lattice, while films cast from benzene and tetrahydrofuran (called film B) showed less crystallinity than film A. As for viscoelastic properties, film A showed peaks of $\tan \delta$ at 38 and 105°C (110 Hz). In the case of film B, the lower-temperature peak was shifted to a higher temperature and the peak was broadened. Film B further exhibited a remarkable decrease of modulus at about 135°C, which was accompanied with a small peak of $\tan \delta$ at this temperature. After being annealed at 140°C, film B showed a remarkable increase in its $\tan \delta$ peak at 105°C and simultaneously the lower-temperature peak shifted to 40°C and the peak at 135°C vanished. To elucidate these changes, differential thermal analysis (DTA) was performed. The DTA thermogram on film B showed an endothermic peak at 135°C, which was not observed after the film was annealed at 140°C. From the fact that the aggregates of superhelices of PBLG, which were precipitated from dimethylformamide solution by adding propionic acid, also showed an endothermic peak at 135°C, it is argued that the superhelical structure exists in the solid state of film B and stacks of benzene ring in the side chain are responsible for the characteristic behaviors of film B.

KEY WORDS Viscoelasticity / Poly(γ -benzyl glutamate) / X-Ray
Diffraction / Differential Thermal Analysis / Solvent Effect /
Superhelix / Stack /

It is well-known that synthetic polypeptides can exist in the form of rigid, rod-like α -helical molecules in solution; this α -helical conformation is in contrast with the random-coil conformation in the solutions of most other synthetic polymers. This observation, together with the fact that synthetic polypeptides show the helix to random-coil transition, stimulated intensive investigations of the dilute solution properties of this class of polymers.

In more concentrated solutions, poly(γ -benzyl-L-glutamate) (PBLG) forms mesomorphic phases akin to cholesteric liquid crystals.¹ In the

mesomorphic phase PBLG molecules are packed approximately parallel, but with a small helical twist relative to one another about an axis perpendicular to the molecular axis. It is easy to envisage that a somewhat similar structure is retained in the solid when all the solvent is removed. X-ray evidence clearly indicates that the helical polypeptide molecules lie in the plane of the film with the helix axes oriented at random in this plane.² Additional observations, including bulk anisotropic swelling of the PBLG films and nuclear magnetic resonance experiments on swollen films, showed that the cholesteric structure of the PBLG liquid crystal is partially retained in the solid state of this

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polymer.³⁻⁵

Nuclear magnetic resonance measurements on solid synthetic polypeptides showed changes in the second moment with temperature which could be accounted for wholly in terms of side-group motion.⁶ The mechanical relaxation behavior of the synthetic polypeptides has also been studied.⁷ McKinnon and Tobolsky⁸ have shown that films of PBLG cast from dimethylformamide (DMF) have two solid-state modifications, which are quite distinct from that cast from chloroform and depend on casting temperature.

In this paper the effects of solvent on the structure and properties of such cast films of PBLG will be reported.

EXPERIMENTAL

The PBLG used in this study was prepared by the polymerization of *N*-carboxyanhydride (NCA) of γ -benzyl glutamate, which was prepared from γ -benzyl glutamate by the established procedure of bubbling phosgene through a 1,2-dichloroethane suspension of the α -amino acid ester. The NCA was reprecipitated from 1,2-dichloroethane (EDC). γ -Benzyl glutamate NCA was polymerized at room temperature in EDC initiated by triethylamine at 40 in *A/I* (anhydride to initiator concentration) ratio. From viscosity measurements in dichloroacetic acid, the molecular weight was estimated to be 170,000.

Films of PBLG about 0.1-mm thick were cast from concentrated solutions (5%) in CH_2Cl_2 , CHCl_3 , EDC, benzene, and tetrahydrofuran (THF). After casting, the films were dried *in vacuo* at room temperature until all traces of solvent were removed. Dynamic mechanical measurements were performed with a Vibron viscoelastometer (Toyo Measuring Instrument Co.), in the temperature range from 20 to 150°C at 110 Hz. To obtain the activation energy, measurements were also carried out at 3.5, 11, and 35 Hz.

Infrared spectra were measured by use of a Jasco spectrophotometer over the region from 400 to 4000 cm^{-1} .

X-ray diagrams were obtained using $\text{CuK}\alpha$ radiation in an X-ray diffraction goniometer manufactured by Rigakudenki Co..

Dilatometric measurements were carried out, using Hg as a confining liquid, at the heating rate of 1°C/3 min. Measurements of density at room temperature were performed by the floatation method, using KBr aqueous solutions containing a small amount of surfactant.

Differential thermal analyses (DTA) were made in a DTA-20B differential calorimeter (Shimadzu Manufacturing Co.). The heating rate was 10°C/min.

RESULTS AND DISCUSSION

Figure 1 shows infrared spectra of thin films of PBLG cast from various solvents. The

Figure 1. Infrared spectra of thin films of PBLG cast from various solvents.

spectra showed peaks at 1650 (amide I), 1550 (amide II), and 600 cm^{-1} (amide V), which indicate a helical conformation. Although the splitting of the amide I band into two peaks shows the presence of a small number of random-coil regions, it is evident that there are no differences in the molecular conformations

Table I. Crystallinities and specific volumes of films cast from various solvents

Solvent	Crystallinity, %	Specific volume, cc/g
EDC	28.7	0.7903
Chloroform	35.2	0.7892
Dichloromethane	33.5	0.7900
THF	8.3	0.7871
Benzene	8.0	0.7863

(a)

(b)

Figure 2. X-ray photographs of PBLG films; (a) cast from EDC, (b) cast from benzene.

among films cast from various solvents. Figure 2 shows X-ray photographs of PBLG films cast from EDC and benzene. An X-ray photograph of a film cast from EDC shows a strong diffraction ring, which has a spacing $d=12.8 \text{ \AA}$ equivalent to that of the (110) plane of a unit cell as given by Bamford, *et al.* A diffuse and weak diffraction ring of the (110) plane in a film cast from benzene shows less crystallinity than the film cast from EDC. In Table I the crystallinities obtained from the X-ray diffraction curves by the usual method are shown with the values of the specific volumes of various films at 17°C. Since the molecular arrangement and the packing of helical molecules in the solid state might be paracrystalline, the values of crystallinities themselves should be considered

Figure 3. E' and $\tan \delta$ as a function of temperature at 110 Hz for PBLG films cast from various solvents; THF (Δ), benzene ($\triangle$), chloroform ($\circ$), EDC ($\bullet$), dichloromethane ($+$).

to illustrate only a relative measure of the ordering of molecules. However, it is interesting to note that films cast from the EDC series (EDC, CH_2Cl_2 , CHCl_3), which are considered to have higher degrees of ordering, have larger specific volumes than the films cast from the benzene series (benzene, tetrahydrofuran). This is considered to be caused by the more compact packing of side chains in the latter.

The experimental results of dynamic mechanical measurements at 110 Hz are shown in Figure 3, where the dynamic modulus E' and $\tan \delta$ are plotted against temperature. Remarkable differences can be seen between the viscoelastic behavior of films cast from the EDC series (called film A), and those from the benzene series (called film B). In the case of film A, $\tan \delta$ shows a peak at 38°C and a corresponding decrease of modulus is also observed around this temperature. This agrees with previous studies and is attributed to the onset of side-group motions. In the case of film B, the temperature of this peak was shifted 10° higher than for the film A and the peak was also broadened. Further, it can be seen that film B

has a much higher modulus than film A at all temperatures between 20° and 130°C . Film B exhibits a large modulus transition at about 135°C , which is accompanied by a small peak in $\tan \delta$ at this temperature. The results of film B are similar to the behavior of form A reported by McKinnon and Tobolsky.⁸ Figure 4 shows the frequency dependence of $\tan \delta$ vs. temperature curves for films A, B, and B annealed at 140°C . It can be seen that another peak exists at 105°C , in addition to the two peaks mentioned above. Despite being less intense in films A and B, this peak noticeably increased in film B after it was annealed at 140°C . Simultaneously, the lower temperature

Table II. Apparent activation energies of the low-temperature dispersion for various films

Sample	Dispersion temperature at 110 Hz, $^\circ\text{C}$	Activation energy, kcal/mol
Film A	38	30
Film B	50	38
Film B after annealing	40	30

Figure 4. Frequency dependence of $\tan \delta$ vs. temperature curves for films cast from EDC, from benzene, and for the film cast from benzene after annealing; 3.5 Hz (+), 11 Hz (▲), 35 Hz (●), 110 Hz (○).

Figure 5. Experimental results of TGA and DTA for films cast from various solvents.

peak shifted to 40°C and the peak at 135°C vanished. Hashino, *et al.*,⁹ assumed the loss maximum at 105°C was caused by segmental motions in the disordered part of the main chains involved as a very small portion of the helical structure; Koleske and Lunberg¹⁰ suggested that films of PBLG contain both paracrystalline and mesomorphic regions and that the relaxation observed near 100°C is indicative of a change from a mesomorphic phase to a paracrystalline one. From infrared absorption measurements the secondary structure of the cast film was almost α -helical and did not vary with casting solvent (Figure 1). It is not clear if the content of the disordered part or mesomorphic region in film B increases after annealing. However, from the different viscoelastic properties of film B, we can conclude that film B must have a structure significantly different from that of film A, while after annealing the structure and properties of film B become similar to those of film A. The apparent activation energies of the side-group motions, calculated from the frequency shift, are given in Table II.

To elucidate the mechanism of the decrease in modulus of film B at 135°C, the thermogravimetric analysis (TGA) and the differential thermal analysis were performed (Figure 5). In the experimental results of TGA, no weight loss was detected from room temperature to

160°C. This shows that thermal degradation did not occur in this temperature range. The DTA thermogram for film B shows an endothermic peak at 135°C, while for film A such a transition was not observed. After being annealed at 140°C, film B did not show the endothermic peak. This means an irreversible change of structure in film B was caused by the heat treatment. In the present stage we can not refer conclusively what kinds of structures were formed in film B. However, it is noteworthy that the aggregates of superhelices of PBLG, which are precipitated from DMF solution by adding propionic acid, also show an endothermic peak at 135°C. In Figure 6 a typical electron micrograph of the superhelical aggregates is shown. Tachibana, *et al.*,¹¹ showed from infrared spectral examination that PBLG in the precipitate had the conformation of an α -helix, the helical aggregates were considered to consist of a coiled coil or superhelix. They further elucidated that the sense of the superhelix of PBG was opposite to the sense of the α -helix, from which the superhelix was made. This relationship had been suggested by Crick¹⁵ in the structure of packing of helices. Ishikawa, *et al.*,¹² showed the X-ray diffraction of superhelical PBLG was very weak except for the reflection of 12.94 Å. Figure 7 shows the X-ray diagram

Figure 6. Electron micrograph of superhelical aggregate of PBLG.

Figure 7. X-ray photograph of superhelical aggregate of PBLG.

of the superhelical aggregates. This diagram is considered to be very similar to that of film B in Figure 2. From this result we can presume that in film B cast from solvents of benzene series, a superhelical structure is formed. Benzene and THF solutions of PBLG are very viscous in comparison with EDC solution. This fact means that PBLG in benzene solution aggregates side-by-side. We presume the molecular aggregates in the solution have a superhelical structure and the film cast from the benzene solution retains the structure existing in the solution. Luzzati, *et al.*,¹³ suggested the presence of coiled coils in the so-called "complex" phase, described by them and found in the system PBLG—DMF from about 15 to 70% (wt/wt) PBLG. Recently, Parry and Elliott¹⁴ in their more detailed examination of this phase have concluded that coiled coils are not present and that the presence of a strong near-equatorial streak, which is one of the main features expected in the diffraction pattern from a coiled coil, does not necessarily demand the presence of a coiled coil structure. They also found that benzyl groups are stacking to produce a quasi-helical arrangement with different periodicity from that of the α -helix of the main chain and that stacks on neighboring molecules link up to form a continuous quasi-

helical side-chain configuration. It is possible that a molecular association caused by the benzene-ring interaction or stack proposed by Parry and Elliott is responsible for the enhanced modulus and higher peak temperature due to side-chain motion in film B. This agrees with the explanation by McKinnon and Tobolsky.⁸ The irreversible transition observed in the thermal measurement of film B is ascribed to the dissociation of the benzene-ring stack in the side chain.

Specific volume *vs.* temperature curves for the films cast from EDC, chloroform, and dichloromethane solution (film A) and from benzene solution (film B) are shown in Figure 8. Film A displays a glass-like transition at about 12°C, which corresponds to the absorption peak of $\tan \delta$ at 38°C (110 Hz), while film B does not clearly show such a transition. Around 95°C another second-order transition is observed for both films A and B. This corresponds to the absorption peak of $\tan \delta$ at 105°C. Film B shows an abrupt change of specific volume in the temperature range from 125° to 135°C and above 135°C the specific volume is consistent with that of film A. This seems to be a first-order transition, as in the melting phenomenon. However the change is not reversible and the cooling curve proceeds along the curves of film A. This is explained by the structural rearrangement accompanying with dissociation of the benzene-ring stack in the side chain of PBLG after being heated above 135°C.

Figure 8. Specific volume *vs.* temperature curves for films cast from various solvents; benzene (●), dichloromethane (▲), chloroform (○), EDC (■).

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