Dynamic Mechanical Properties of Poly(α-amino acid)s. II. Poly(ε-N-benzyloxycarbonyl-L-lysine)

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ABSTRACT: Dynamic mechanical measurements for solid films of poly(ε-N-benzyloxycarbonyl-L-lysine) (PCLL) were carried out with torsion pendulum apparatus over a frequency range from 1.0 to 0.003 Hz at various temperatures within its side-chain dispersion region, and the effects of annealing were also examined. Frequency dispersion curves of real and imaginary parts of complex rigidity and loss tangent were superposed onto master curves by performing suitable shifts along the logarithmic frequency axis. The temperature dependence of shift factor a_T was well represented by the Williams—Landel—Ferry equation with the universal parameters. The dispersion exhibits some characteristics of the glass transition, as was found for the side-chain dispersions of poly(γ-benzyl L-glutamate) (PBLG), poly(γ-methyl L-glutamate) (PMLG), and their copolymers. The relaxation spectrum for PCLL is considerably sharper than those for PBLG or PMLG. As the temperature of the annealing is increased, the dispersion shifts to higher temperatures (longer times) and the distribution of the relaxation times becomes a little broader.

KEY WORDS   Poly(ε-N-benzyloxycarbonyl-L-lysine) / Side-Chain Dispersion / Time—Temperature Superposition / WLF Equation / Relaxation Spectra / Annealing /

Solid state properties of poly(α-amino acid)s have been investigated with various physical means such as dynamic mechanical, dielectric, NMR, X-ray, dilatometric, etc. It has been found for several poly(α-amino acid)s that a transition associated with the onset of the side-chain rotation appears, and that the transition exhibits certain characteristics of a glass transition. It was quite possible for the temperature and frequency dependences of the dynamic mechanical[1] and dielectric[2] behaviors of poly(γ-benzyl L-glutamate) (PBLG), poly(γ-methyl L-glutamate) (PMLG), and their copolymers to be represented by the universal Williams—Landel—Ferry (WLF) equation,[3] while the side-chain dispersion of other synthetic amorphous polymers, for example, poly(n-alkyl methacrylate)s are known to obey simple Arrhenius type equation.[4-7] An X-ray study suggested that the transition occurs obviously in the crystalline region and is, nevertheless, analogous to the glass transition.[8]

The discontinuity in the slope of the specific volume—temperature curve was observed for PBLG and PMLG near room temperature.[9-12] In these polymers, the backbone chain is in the α-helix conformation and the side chains form a soft shell of considerable thickness around the helical core, which is denoted as the side-chain region. The break-point temperature and the thermal expansion coefficients of the side-chain region, estimated on the assumption that the change in the thermal expansion coefficient of the core is neglegible, are close to the values expected from the empirical equations proposed for the glass transition.[1,9]

For poly(ε-N-benzyloxycarbonyl-L-lysine) (PCLL), which has longer side chains than PBLG or PMLG, a very strong dynamic mechanical loss[13] and a sharp decrease in the NMR second moment[14] have been noted in the temperature range just above room temperature, and are attributed to the side-chain motion. These previous investiga-

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tions are based on isochronous measurements, which are insufficient for discussing the mechanism of the relaxation and the relation with the structure. Therefore, isothermal measurements over a wide frequency range are necessary.

Solid state properties of poly(α-amino acid)s are markedly affected by the conditions of sample preparation; casting solvent,[11,15—18] thermal history,[19—21] etc.

In this paper, the temperature and frequency dependences of the complex rigidity of PCLL are studied. The effects of annealing were also examined. The results are discussed in terms of structure and motions of side chains.

EXPERIMENTAL

The PCLL used in this study was prepared by polymerization of its α-amino acid NCA in dioxane in the presence of a trace of triethylamine as an initiator. The molecular weight of the polymer was estimated as about 280,000 from the limiting viscosity number in m-cresol. Films of PCLL were cast from a concentrated dimethylformamide (DMF) solution on a glass plate at a temperature of about 50—60°C. After being dried, some of these were immersed in methanol for few days to remove residual solvent, then dried, and annealed for 24 h *in vacuo* at following temperatures prior to each run of dynamic mechanical measurements: samples a: 84°C, b: 112°C, c: 135°C, and d: 160°C. Sample f was annealed at 190°C *in vacuo* for 3 days, and sample e, not immersed in methanol, was annealed at 160°C.

Dynamic mechanical measurements were carried out with torsion pendulum apparatus in a mode of forced oscillation[22] over an ultra-low frequency range of 0.003 to 1.0 Hz at temperatures between 10 and 80°C.

X-ray photographs were obtained on a flat-film camera using Cu—K$_\alpha$ radiation with the incident beam parallel to the surface of the specimen. Camera distance was calibrated using Si powder coated around the specimen.

RESULTS AND DISCUSSION

Figure 1 shows the temperature dependence of the real part of complex rigidity G' and loss tangent tan δ at a frequency of 0.03 Hz for PCLL. The
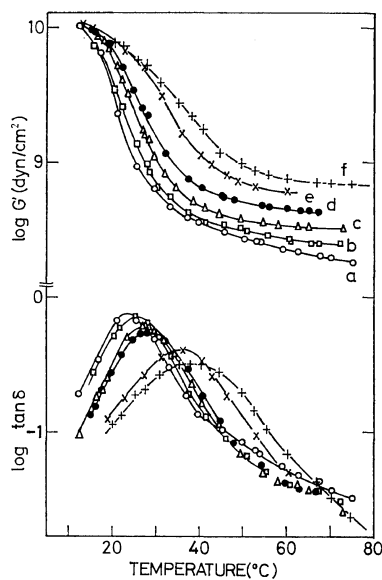

Figure 1. Real part of complex rigidity and loss tangent for solid films of PCLL annealed at various temperatures.

viscoelastic properties of PCLL are influenced apparently by the annealing conditions. A loss peak of the side-chain dispersion[13,14] is observed at temperatures of 20—40°C depending on the thermal history of the specimen. The peak moves to higher temperatures and decreases in magnitude as the annealing temperature rises.

Figures 2 and 3 show the frequency dependence of real and imaginary parts of complex rigidity, G' and G'', and tan δ for sample a at various temperatures between 5.0 and 54.0°C, and for sample e between 13.0 and 60.5°C, respectively. The side-chain dispersion is observed fragmentarily at each temperature. Similar measurements were carried out for all the samples.

The frequency dispersion curves of G', G'', and tan δ for each sample obtained at various temperatures in the side-chain dispersion region were superposed onto the respective master curves by performing suitable horizontal translations along the logarithmic frequency axis.

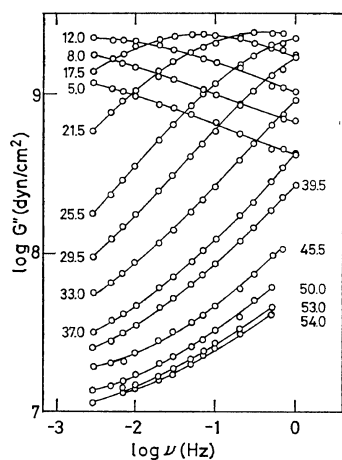The principle of time—temperature superposition is expressed as follows.[23]

$$G'(\omega) = G_0'(\omega a_T)$$
$$G''(\omega) = G_0''\omega(a_T)$$

and

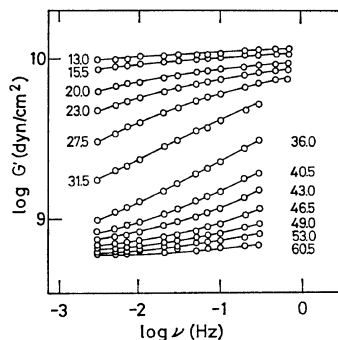$$\tan \delta(\omega) = \tan \delta_0(\omega a_T)$$

$$(1)$$
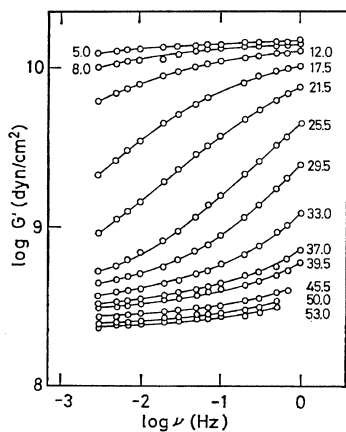
(a)

(b)
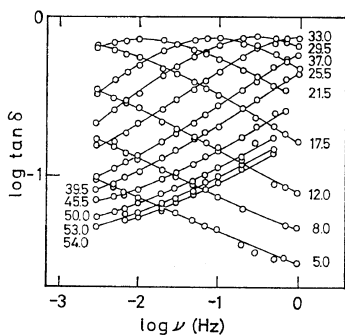
(c)

Figure 2. Variation of real (a) and imaginary (b) parts of complex rigidity and loss tangent (c) with frequency for sample a at temperatures between 5.0 and 54.0°C.
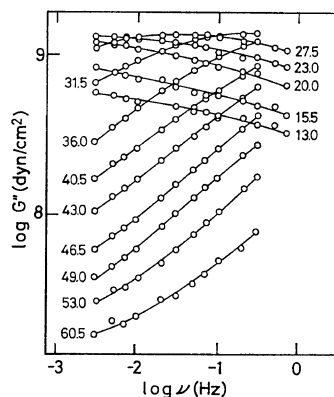

(a)

(b)
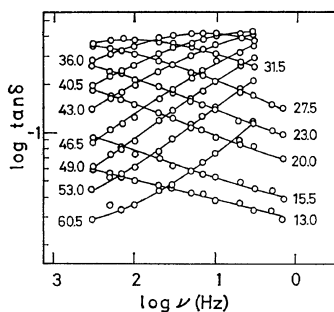

(c)

Figure 3. Variation of real (a) and imaginary (b) parts of complex rigidity and loss tangent (c) with frequency for sample e at temperatures between 13.0 and 60.5°C.

where the subscript zero refers to a value at a reference temperature T_0, ω is the angular frequency, and a_T is the shift factor. Log a_T gives the amount of horizontal translation along the loga-

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rithmic frequency axis. The correction factor $\rho_0 T_0/\rho T$, resulting from thermal expansion and rubber-like character of the elasticity, was assumed to be unity in this case.

The composite relaxation curves of G', G'', and $\tan \delta$ for sample a, which were reduced to a temperature of 37°C, composed from the curves given in Figure 2, are shown in Figure 4. Figure 5 shows the composite relaxation curves for sample e reduced to a temperature of 36°C, composed from the curves given in Figure 3. The side-chain dispersion is observed over more than ten decades of frequency. The superposition seems fairly applicable over the whole frequency range.

Figure 6 shows the composite relaxation curves for all the samples examined. The curves are reduced to a temperature of 37°C. It can be seen
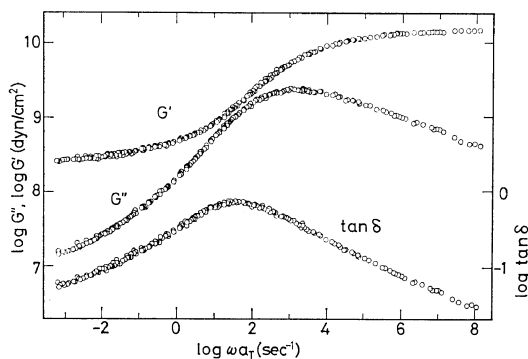

Figure 6. Composite relaxation curves of real and imaginary parts of complex rigidity and loss tangent reduced to 37°C for solid films of PCLL annealed at various temperatures.

Figure 4. Composite relaxation curves of real and imaginary parts of complex rigidity and loss tangent for sample a reduced to 37°C.
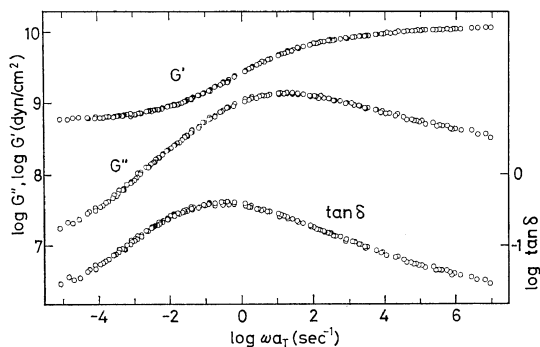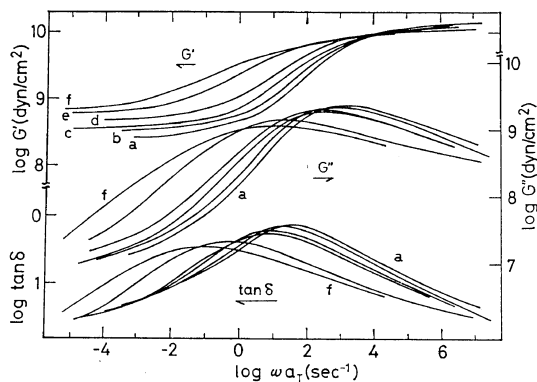

Figure 5. Composite relaxation curves of real and imaginary parts of complex rigidity and loss tangent for sample e reduced to 36°C.
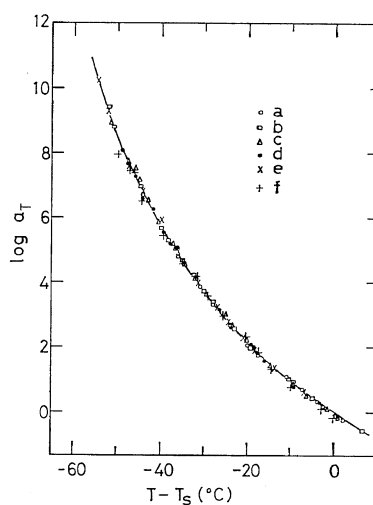
Figure 7. Shift factor $\log a_T$ plotted against $T-T_S$ for solid films of PCLL annealed at various temperatures. The solid curve represents universal WLF equation, eq 2.

that the peaks of G'' and $\tan \delta$ move to lower frequencies by increasing the annealing temperature, and that the value of G' at lower frequencies increases by about three times, while it is hardly changing at higher frequencies.

The logarithm of the shift factor $\log a_T$, required for the superposition procedure, is plotted in Figure 7. The temperature dependence of $\log a_T$ is represented well by the universal WLF equation[3]

$$\log a_{\mathrm{T}} = \frac{-8.86(T-T_{\mathrm{s}})}{101.6+T-T_{\mathrm{s}}} \qquad (2)$$

with an appropriate choice of T_{s} for each sample, as has been found for the side-chain dispersion of PBLG, PMLG, and their copolymers.[1,2] The reference temperature T_{s} of the universal WLF equation appreciably increases by increasing the annealing temperature. The values for T_{s} are listed in Table I.

Table I. Annealing temperatures T_{a}, reference temperatures of WLF equation T_{s}, and spacings of the samples

sample	T_{a}, °C	T_{s}, °C	d_{100}, Å
a	84.0	57.0	14.44
b	112.0	60.5	14.35
c	135.0	64.0	14.28
d	160.0	64.0	14.24
e[a]	160.0	67.5	13.83
f[b]	190.0	70.5	13.62

[a] Not immersed in methanol.

[b] Annealed for 3 days.

The WLF equation is well known for describing the temperature dependence of mechanical or dielectric relaxation times in the glass transition region for a wide variety of glass-forming substances including amorphous and semicrystalline polymers.

Pezzin, et al.,[24] investigated DSC behavior for several poly(α-amino acid)s including PCLL in the side-chain transition region, and found that the poly(α-amino acid)s exhibit all the characteristics of the glass transition.

A volume—temperature relation for PCLL, examined in detail,[9] had revealed a break point associated with the onset of side-chain motions at 8°C for a sample annealed at 100°C. This breakpoint temperature may be regarded as the glass transition temperature T_{g}. For samples a and b, annealed at 84 and 112°C, respectively, this T_{g} value is close to the one expected from the empirical relation $T_{\mathrm{s}} - T_{\mathrm{g}} = 50 \pm 5$°C.[3]

Thus, it is concluded, as we did for PBLG and PMLG in the previous papers,[1,2] that the side chain of PCLL has a disordered conformation and is randomly packed in inter-helix region. It is likely that side chains, microscopically segregated from main backbone chain, undergo motion and
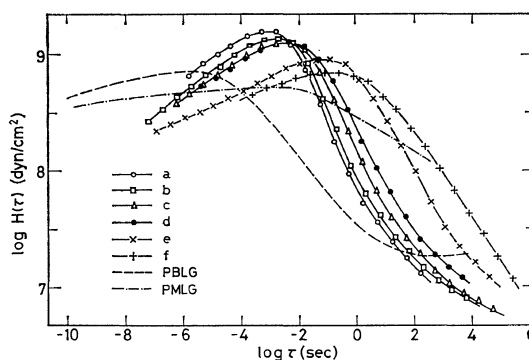

Figure 8. Relaxation spectra reduced to 37°C for solid films of PCLL. The spectra for PBLG (------) and for PMLG (–·–·–) are also included.

exhibit behavior similar to the glass transition independently of the main chain in the α-helix structure.

Relaxation spectra were calculated from the master curves of G' and G'' in Figure 6, using the second approximation method of Williams and Ferry.[25] For each of the samples, the results obtained separately from the real and imaginary components of experimental data are in close agreement. The spectra are shown in Figure 8, and compared with those for PBLG and PMLG.[1]

It can be seen that the distribution of relaxation times becomes narrower and the intensity increases as the length of side chain increases in the order of PMLG, PBLG, and PCLL, though the spectra for PCLL change depending on the conditions of annealing. The spectrum for PMLG is very broad, like those for semicrystalline polymers or the β-spectra of poly(n-alkyl methacrylate)s below glass transition temperatures.[5,7] Since the side chains of these poly(α-amino acid)s stick out from the rigid α-helical backbone and are accomodated in inter-helix regions, they should behave in a fashion similar to polymer segments of semicrystalline polymers in amorphous layers sandwiched between crystalline regions. Fujino, et al.,[26,27] investigated the effects of successive change in morphology from amorphous to crystalline upon mechanical behavior with a series of polymers, partly to completely acetylated, and partly to highly formalized poly(vinyl alcohol). They found that the slope of the wedge portion of the relaxation spectrum decreased as the degree of acetylation or formalization of the polymer decreased $i.\ e.$, with increasing the crystallinity of the polymer. The increase in

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side-chain length of poly(α-amino acid)s corresponds to the increase in fraction of amorphous region. It is likely that as the length and flexibility of the side chain increase, motions of the side chain become more and more independent of the backbone and the spectrum becomes sharper.

As the annealing temperature is increased, the relaxation spectrum of PCLL moves to longer times, decreases in intensity, and distribution of relaxation times becomes a little broader. The negative slope of the wedge portion (longer time region) decreases from 0.6 (sample a) to 0.4 (sample f). This, together with the results shown in Figures 1 and 6 and variation in the value of T_g, imply that motions of side chains are more restricted by annealing at higher temperatures.

The effects of annealing on side-chain relaxation have been reported for PMLG.[19-21] Kajiyama, et al.,[21] found for PMLG films cast from dichloroethane solution that the degree of crystallinity increases and also that the α-helix chains in the crystalline region are packed more closely by increasing the annealing temperature, resulting in a higher temperature shift of the γ relaxation which arises from the motions of the side chains of the α-helix in the crystalline region.

Similar results were obtained in the present case except for the change in crystallinity. Figure 9 shows X-ray photographs of samples a and f. The degree of crystallinity of PCLL apparently decreases by annealing as seen in the photographs. As the rod-like molecules are packed almost parallel even in the noncrystalline mesomorphic region and the side chains are randomly arranged even in

the crystalline (or paracrystalline) region, the degree of the crystallinity seems not to have a major effect on the side-chain mobility.

As the annealing temperature is increased, spacings of (100), (110), and (200) reflections gradually decrease. The spacing of (100) reflection d_{100} is listed in the last column of Table I. The inter-helix distance for sample f is smaller than that of sample a by about 6%. This corresponds to a decrease in the volume of the side-chain region by about 14%, assuming no change in the volume of the core of the α-helix.

The α-helix cores of the neighboring molecules do not directly interact because of the existence of the long and flexible side chains, which form a shell of considerable thickness. Thus, the contraction of the spacings is likely due to an interaction among the side chains.

IR absorption spectrum of a thin film of PCLL cast from DMF solution showed peaks of amide I and amide V bands at 1665 and 620 cm^{-1}, respectively, indicating the α-helix conformation of the peptide chain. Annealing at a temperature upto 190°C does not change the backbone conformation.

Hydrogen-bonded NH stretching band at 3300 cm^{-1} is accompanied with a shoulder of free NH band at 3410 cm^{-1}. Since the backbone is in the α-helix conformation, the shoulder should be attributed to the side-chain urethane groups. Hatano and Yoneyama[28] have suggested that 38% of the urethane groups of the side chain form intramolecular hydrogen bonds in chloroform solution, assuming that the absorption coefficients of all the
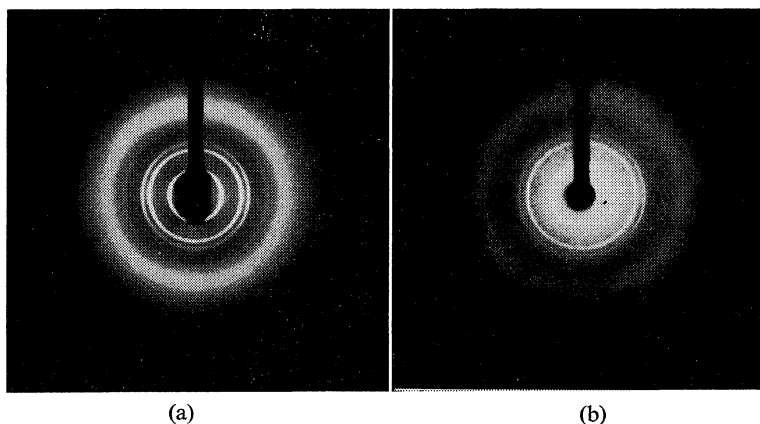

Figure 7. X-Ray photographs for solid films of PCLL: (a) sample a; (b) sample f.

NH-stretching vibrations in the system are equal. In the solid state, the urethane groups form intermolecular hydrogen bonds as well as intramolecular ones. The fraction of the hydrogen-bonded NH groups of the side chain was roughly estimated as 82% from ratio of the absorption area of the two bands.[29] In fact, the side-chain dispersion region of PCLL almost coincides with those of α-dispersion of poly(urethane)s,[30] of which urethane groups are almost completely hydrogen bonded even in the amorphous region.[31]

The nature of the hydrogen bonds occurring in the polyamides and the polyurethanes in the solid state has been studied.[31-33] Jeffries used the IR-deutration technique to investigate the molecular order of nylon 66, which had been melted and then quenched to 0°C. He showed that the strength and regularity of the amide—amide interactions in the nylon structure vary depending upon the temperature and duration time in the molten state.[32]

The effect of annealing observed for PCLL probably arises from the change in the number and/or strength of the urethane—urethane hydrogen bonds in the side-chain region.

CONCLUSIONS

It was possible for the frequency dispersion curves of PCLL obtained at various temperatures in the side-chain dispersion region to be closely superposed onto master curves by applying a horizontal shift along the logarithmic frequency axis. The temperature dependence of the shift factor follows the universal WLF equation. The relaxation spectrum has a negative slope of about 1/2 at longer relaxation times (wedge portion). By increasing the annealing temperature, the packing of the α-helix molecules becomes denser. The dispersion shifts to higher temperatures (longer relaxation times) and the distribution of relaxation times becomes a little broader. This may be attributed to the change in the number and/or urethane groups.

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