Dielectric Dispersion of Poly(ϵ -*N*-carbobenzoxy L-lysine) and Related Poly(α -amino acid)s in the Solid State

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ABSTRACT: Dielectric measurements were performed for poly(ϵ -*N*-carbobenzoxy L-lysine) (PCLL), a blended sample of PCLL and poly(γ -benzyl D-glutamate) (PBDG) (PCLL + PBDG), and a copolymer of ϵ -*N*-carbobenzoxy L-lysine and γ -benzyl L-glutamate (PCLBG). In each sample, a dielectric dispersion due to the onset of large scale motion of the side chain was observed at about 50°C. These dispersion temperatures were higher than that of PBLG. This is contrary to the expectation that the increasing degree of freedom of motion due to the side chain of the CLL-residue, which is longer than that of the BLG-residue, may lower the dispersion temperature. A strong interaction was assumed to exist between side chains of PCLL. Infrared spectroscopic measurements revealed that the urethane groups in CLL-side chains are hydrogen-bonded. Fractions of hydrogen bonds of the side-chains of PCLL, PCLL + PBDG, and PCLBG were estimated from the results of infrared spectroscopy. There is a qualitative agreement with the results of dielectric measurements.

KEY WORDS Poly(ϵ -*N*-carbobenzoxy L-lysine) / Dielectric Dispersion / Side-Chain Motion / Hydrogen Bonds /

For many years, poly(α -amino acid) has been used as a simple model in the theoretical and experimental studies of the interaction which determines the three-dimensional conformation of proteins. Thus, a considerable body of experimental data on the molecular motions of poly(α -amino acid)s has been accumulated. It is well known that the long side chain of poly(α -amino acid) shows a considerable mobility above an appropriate temperature, though no remarkable motion of the main chain is observed.¹⁻⁹

Recent investigations in this laboratory have shown that the transition associated with the onset of side-chain motion exhibits some characteristics of a glass transition for several poly(α -amino acid)s.^{10,11} In our previous reports,¹²⁻¹⁴ the behavior of the side-chain dispersion of poly(ϵ -*N*-carbobenzoxy L-lysine) (PCLL) was found to be well described by the WLF-equation, as is true in

the case of poly(γ -benzyl L- or D-glutamate) (PBLG or PBDG) and poly(γ -methyl L-glutamate) (PMLG).^{10,11}

The relationship between the length of the side chain of poly(alkyl glutamate)s and the side-chain mobility has been studied.^{15,16} The increase in the side-chain length was found to enhance the side-chain mobility. Thus, it may be expected that the dispersion temperature of PCLL might be far below those of PBLG and PMLG, because the side chain of PCLL is much longer than those of PBLG and PMLG. Contrary to this expectation, the dispersion temperature of PCLL is higher than those of PBLG and PMLG.¹⁰⁻¹⁴

In this work, in order to explain this unexpected fact, dielectric measurements were performed for PCLL, a mixture of PCLL and PBDG (PCLL + PBDG), and a copolymer of ϵ -*N*-carbobenzoxy L-lysine and γ -benzyl L-glutamate (PCLBG). Infrared absorption spectra were also obtained for these samples.

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The results are discussed in the light of the interaction between side chains which is expected to exist for the particular polymers studied.

EXPERIMENTAL

PCLL and PCLBG were prepared by polymerizing their respective NCA's in dioxane with a trace of triethylamine as an initiator. To obtain PCLBG, equi-moles of CLL—NCA and BLG—NCA were polymerized. The ratio of BLG- and CLL-residues in the copolymer was determined by the amino acid analysis and was found to be BLG: CLL=2:1. PCLL+PBDG was prepared by dissolving equi-moles of PCLL and PBDG in *N,N*-dimethylformamide (DMF). Films for measurements were cast from concentrated DMF solutions on a glass plate at 50°C. Films were painted with conductive silver paste in order to assure complete contact with the electrodes. Each film was set in the measuring cell and evacuated at a temperature of 120–150°C until the dielectric constant at the temperature became constant.

The apparatus and procedure for dielectric measurements have been described previously.^{10,17} Measurements were made over the frequency range from 110Hz to 1MHz. The temperature was changed from –150°C to 100°C.

Films for measurements of infrared absorption spectra were obtained by evaporation to dryness of viscous DMF solutions. The orientation was produced by unidirectional shearing of the viscous solution. The best oriented film was chosen after examination under a polarizing microscope. The infrared absorption spectra were recorded on a Hitachi EPI-G3 spectrometer.

RESULTS AND DISCUSSION

Figure 1 shows the temperature dependence of the dielectric constant ϵ' and the loss factor ϵ'' of PCLL, PCLL+PBDG, and PCLBG, measured at 1kHz. In each sample, two dielectric dispersions were observed, at about 50°C and –70°C.

According to previous NMR⁹ and dynamic mechanical measurements^{12,13,18} for PCLL, the dispersion observed near room temperature is considered to be caused by the micro-Brownian motion

Figure 1. Temperature dependence of the dielectric constant ϵ' (a) and the loss factor ϵ'' (b) for PCLL (○), PCLL+PBDG (●), and PCLBG (⊙) at 1 kHz.

of the whole side chain, and the dispersion observed far below room temperature is due to the oscillational motion of the side chain on a small scale.^{19,20} The dielectric dispersion observed for PCLL+PBDG and PCLBG is assigned to the same origin as PCLL. In this paper, we will discuss only the dispersion near room temperature.

Figure 2 shows the frequency dependence of ϵ' and ϵ'' for PCLL at various temperatures from 40.0°C to 85.5°C. All of these ϵ' - and ϵ'' -frequency curves could be superposed, after normalization with the relaxation strength $\epsilon_0 - \epsilon_\infty$, on a set of frequency dependent curves at a certain temperature by performing suitable horizontal transla-

Figure 2. Frequency dependence of the dielectric constant ϵ' (a) and the loss factor ϵ'' (b) for PCLL at the indicated temperatures.

tions along the logarithmic frequency axis.

In Figure 3, a set of composite master curves for PCLL obtained in this way is shown. Figures 4 and 5 also show composite master curves for PCLL+PBDG and PCLBG, respectively. In each sample, the time-temperature superposition principle is found to be well applicable. The shift factors b_T of PCLL+PBDG and PCLBG as well as PCLL were well described by the universal WLF-equation. Master curves for ϵ'' of PCLL+PBDG and PCLBG show a single peak and are as sharp as that of PCLL. This implies that the side chains of the CLL-residue and the BLG- (or BDG-) residue of PCLL+PBDG and PCLBG undergo motions cooperatively.

Table I shows the dispersion temperature $T_{\max}$,

Figure 3. Composite dispersion curves of the normalized dielectric constant ($\oplus$) and loss factor ($\circ$) for PCLL reduced to 61.5°C. b_T is the shift factor.

Figure 4. Composite dispersion curves of the normalized dielectric constant ($\oplus$) and loss factor ($\circ$) for PCLL+PBDG reduced to 60.5°C. b_T is the shift factor.

Figure 5. Composite dispersion curves of the normalized dielectric constant ($\oplus$) and loss factor ($\circ$) for PCLBG reduced to 48.0°C. b_T is the shift factor.

the temperature of the loss maximum at a frequency of 1kHz, of PCLL, PCLL+PBDG, and PCLBG with that of PBLG for comparison. Dis-

Table I. Dielectric dispersion temperature

Sample	$T_{\max}$, °C
PCLL	55
PCLL+PBDG	55
PCLBG	43
PBLG	30 ^a

^a Ref 10.

persion temperatures of all samples measured here are obviously higher than that of PBLG. Thus, in the vicinity of room temperature, the motion of the side chains in these samples is remarkably restricted, compared with that of the PBLG side chain. It is probable that there exists a strong interaction among the CLL-side chains.

Figure 6 shows infrared absorption spectra of oriented films of PCLL, PCLL+PBDG, and PCLBG. In the NH-stretching absorption region of these polymers, two bands are observed: a free NH-band at 3400 cm^{-1} and a hydrogen-bonded NH-band at 3300 cm^{-1} . Two species are responsible for the free NH-stretching absorption band: one is the side-chain amide, the other is the amide group of the main chain where the α -helix is locally broken. The backbone chains of these polymers, however, are thought to be almost perfect α -helixes. Since some evidence of the higher mobility of the side chain as compared with that of the main chain has been accumulated,^{10,11} it may be reasonable to assign the free NH-stretching band to the *N*-carboxy amino (ure-

Figure 6. Polarized infrared absorption spectra of (a) PCLL, (b) PCLL+PBDG, and (c) PCLBG: ----, electric vector perpendicular to orientation direction; —, electric vector parallel to orientation direction.

thane) group of the side chain.

Figure 7 shows infrared absorption spectra of PCLBG at various temperatures from 23°C to 101°C. The free NH-stretching band at 3400 cm^{-1} becomes important with increasing temperature. Dielectric measurements showed that in this temperature range the side chain of PCLBG undergoes considerable motion while the main chain does not. Thus, the increase in intensity of the free NH-stretching band appears to be related to the side-chain motion.

It is well known for polyamide and polyurethane that the carboamide groups strongly interact through hydrogen bonds with neighboring molecules and that the networks of such hydrogen bonds play an important role in the physicochemical properties.²¹ Hatano and Yoneyama reported that for poly(amino acid)s having a urethane group in the side chain, such as for PCLL, poly(δ -*N*-carbobenzoxy L-ornithine), and poly(γ -*N*-carbobenzoxy L- α , γ -diaminobutyric acid), the urethane group is hydrogen-bonded with those in the neighboring side chains in chloroform—dichloroacetic acid solution.²² It is natural to consider the hydrogen bond among the side chains of polymers studied here even in the solid state. Thus, the hydrogen-bonded NH-stretching absorption band at 3300 cm^{-1} is contributed from both the backbone and the side chain.

On the assumption that absorption coefficients

Figure 7. Infrared absorption spectra for PCLBG at indicated temperatures. The electric vector is perpendicular to the orientation direction.

of the NH-stretching vibrations at 3400 cm^{-1} and 3300 cm^{-1} are equal, the mole fractions of the hydrogen-bonded NH-groups to all the urethane group of the side chain were estimated to be 82, 61, and 19%, for PCLL, PCLL+PBDG, and PCLBG, respectively.

Hatano and Yoneyama reported that the mole fraction of the hydrogen-bonded NH groups for PCLL in chloroform—dichloroacetic acid solution

is 38%.²² The mole fraction of the hydrogen-bonded NH-group in the solid state is much larger than that in solution, beyond experimental error, suggesting that in the solid state a certain number of hydrogen bonds are formed between side chains of neighboring molecules. Furthermore, these results may imply that in the case of PCLL+PBDG, hydrogen bonds are possible between the urethane group in the side chain of the CLL-residue and the carbonyl group in the side chain of the BDG-residue.

When the polar urethane NH group is hydrogen-bonded, the side-chain motion will be so hindered that the dipole cannot orient with the applied electric field. Thus, the dielectric constant ϵ' observed will be diminished upon increasing the mole fraction of hydrogen-bonded NH-groups.

As seen in Figure 1a, the dielectric constant in the vicinity of room temperature for PCLL, PCLL+PBDG, and PCLBG are 3, 4, and 5, respectively. Thus, the mole fraction of hydrogen-bonded NH-groups in the side chain expected from the dielectric constant ϵ' is in the order of PCLL > PCLL+PBDG > PCLBG.

This relation accords well with the relation among the mole fractions of hydrogen bonds estimated from infrared absorption spectra. The qualitative agreement of the fraction of hydrogen bonds deduced by two independent methods enhances the reliability of the idea of the existence of hydrogen bonds in the side chain of PCLL in the solid state.

As noted in Table I, the dielectric dispersion temperature is found to rise upon increasing the fraction of hydrogen bonds. It may be the hydrogen bond in the side chain that causes the higher dielectric-dispersion temperature of PCLL in the solid state, compared with those of PBLG and PMLG.

CONCLUSIONS

The dielectric dispersion of PCLL, PCLL+PBDG, and PCLBG at about 50°C due to the onset of the side-chain motion implies the existence of strong interaction in these polymers. Infrared absorption spectra revealed that there should be hydrogen bonds among the urethane groups of the CLL-side chains. The relation of the dielectric constant ϵ' , PCLL(3) < PCLL+PBDG(4) <

PCLBG(5), in the vicinity of room temperature was well explained in terms of hydrogen bonds in the side chains of these polymers studied by infrared absorption spectra. The dispersion temperatures were also found to be related to the fraction of hydrogen bonds. The existence of hydrogen bonds in the side chain was found to be one of the important factors in understanding the properties of PCLL and the related poly(α -amino acid)s in the solid state.

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REFERENCES

1. R. G. Saba, J. A. Sauer, and A. E. Woodward, *J. Polym. Sci., Part A*, **1**, 1843 (1963).
2. H. Obata and S. Ogawa, *Rep. Prog. Polym. Phys. Jpn.*, **81**, 121 (1965).
3. Y. Hashino, N. Yoshino, and K. Nagamatsu, *ibid.*, **8**, 221 (1965).
4. Y. Hashino, M. Yoshino, and K. Nagamatsu, *ibid.*, **9**, 297 (1966).
5. K. Hikichi, K. Saito, M. Kaneko, and J. Furuichi, *J. Phys. Soc. Jpn.*, **19**, 557 (1964).
6. S. Makino, K. Kamashima, S. Kubota, and S. Sugai, *Jpn. J. Appl. Phys.*, **3**, 55 (1964).
7. J. A. E. Kail, J. A. Sauer, and A. E. Woodward, *J. Phys. Chem.*, **66**, 1292 (1963).
8. K. Hikichi, *J. Phys. Soc. Jpn.*, **19**, 2169 (1964).
9. A. Tsutsumi, *Jpn. J. Appl. Phys.*, **9**, 2225 (1970).
10. A. Tsutsumi, K. Hikichi, T. Takahashi, Y. Yamashita, N. Matsushima, M. Kanke, and M. Kaneko, *J. Macromol. Sci.-Phys.*, **B8**, 413 (1973).
11. Y. Yamashita, A. Tsutsumi, K. Hikichi, and M. Kaneko, *Polym. J.*, **8**, 114 (1975).
12. Y. Yamashita, A. Tsutsumi, K. Hikichi, and M. Kaneko, *Rep. Prog. Polym. Phys. Jpn.*, **18**, 521 (1975).
13. Y. Yamashita, A. Tsutsumi, K. Hikichi, and M. Kaneko, *ibid.*, **19**, 513 (1976).
14. N. Sasaki, A. Tsutsumi, K. Hikichi, and M. Kaneko, *ibid.*, **19**, 521 (1976).
15. S. Sugai, K. Kamashima, S. Makino, and J. Noguchi, *J. Polym. Sci., Part A-2*, **4**, 183 (1966).
16. Y. Yokomori, Y. Uematsu, and I. Uematsu, *Rep. Prog. Polym. Phys. Jpn.*, **15**, 633 (1972).

17. T. Takahashi, A. Tsutsumi, K. Hikichi, and M. Kaneko, *Macromolecules*, **7**, 806 (1974).
18. A. Hiltner, J. M. Anderson, and E. Borkowski, *ibid.*, **5**, 446 (1972).
19. K. Yamafuji, *J. Phys. Soc. Jpn.*, **15**, 2295 (1960).
20. R. Hayakawa and Y. Wada, *J. Polym. Sci., Polym. Phys. Ed.*, **12**, 2119 (1974).
21. N. G. McCrum, B. E. Read, and G. Williams, "Anelastic and Dielectric Effects in Polymer Solids," Wiley and Sons, London, 1967.
22. M. Hatano and M. Yoneyama, *J. Am. Chem. Soc.*, **92**, 1392 (1970).