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Side-Chain Interactions in the *α*-Helical Form of Poly(*ε*-*p*-Toluenesulfonyl L-Lysine)

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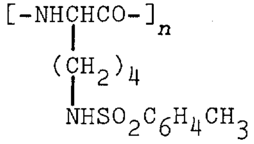
SUMMARY

Poly(ε-*p*-toluenesulfonyl L-lysine) was synthesized by the N-carboxyanhydride method. The molecular conformation was the right-handed α-helix in helicogenic solvents and in the solid state. The crystal structure was hexagonal with the unit-cell dimension a = 1.680 nm and the unit height h = 0.1498 nm. The x-ray structure analysis clarified that the side chains intervened mutually between neighboring helices. The glass transition temperature associated with the side-chain motion was about 60°C and much higher than those of similar polypeptides. The thin solid film exhibited the extrinsic circular dichroism due to the side chain, which was induced by a chiral array of the side chains.

INTRODUCTION

Poly(α-amino acid)s can assume the rodlike α-helical conformation in a variety of solvents and in the solid state. It is well known that the physical properties are modified by introducing a functional group into the side chain.

In a previous study (SASAKI et al. 1983), some of the present authors have shown that the urethane groups in the side chains in α-helical poly(δ-carbobenzoxy L-ornithine) are associated by hydrogen bonding. In this paper, we discuss the crystal structure and the solid-state properties of poly(ε-*p*-toluenesulfonyl L-lysine) (PTLL).



$$\begin{array}{l}[-\mathrm{NHCHCO}-]_n\\ \qquad\;|\\ \qquad(\mathrm{CH_2})_4\\ \qquad\;|\\ \qquad\mathrm{NHSO_2C_6H_4CH_3}\end{array}$$

The side chain of this polymer is bulky and has a large dipole moment of 5.4 D (LE FÈVRE and VINE 1938).

EXPERIMENTAL

The monomer, ε-*p*-toluenesulfonyl L-lysine was prepared by the method described by Erlanger et al. (1959), and polymerized by the N-carboxyanhydride method using triethylamine as the initiator in dioxane. The polymer was identified by its NMR (Fig. 1) and infrared spectra. The infrared amide bands characteristic of the α-helical conformation were observed at the wavenumbers of 3288 (amide A), 1651 (amide I), 1546 (amide II), and 615 cm^{-1} (amide V). The Moffitt-Yang parameter b_0 = -560, observed for a 1% solution in chloroform, suggested the right-handed helical conformation. Oriented films were prepared by using a hot press at 100°C from films obtained by casting solutions in chloroform.

X-ray diffraction measurements were carried out with Ni-filtered Cu Kα radiation using a cylindrical camera. Reflection spacings were calibrated against reflections of silicon powder sprinkled over the specimen. Intensities of equatorial reflections were measured by microphotometer scans of photographs and corrected for the Lorentz-polarization factor and the multiplicity.

The circular dichroism (CD) was recorded for thin films prepared on a quartz plate. The effect of the birefringence due to the molecular orientation was insignificant, since the CD spectra were not changed by the rotation of the plate in its plane.

Differential scanning calorimetric (DSC) measurements were carried out at a scanning rate of 10°C/min.

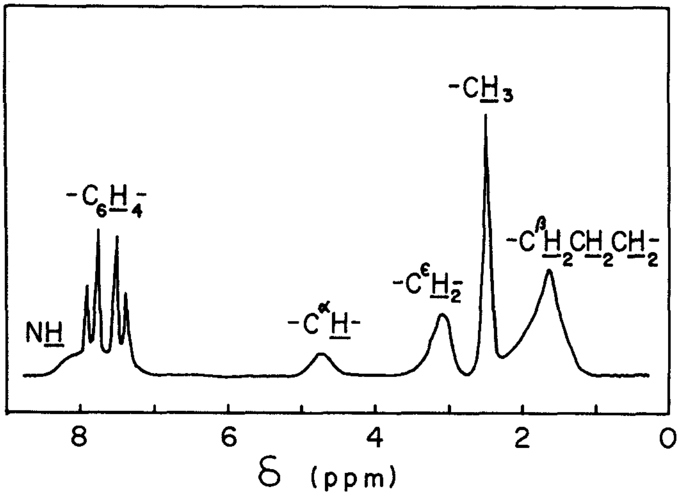


Fig. 1. NMR spectrum of PTLL measured for the solution in trifluoroacetic acid.

RESULTS AND DISCUSSION

Fig. 2 shows an x-ray diffraction photograph of the oriented sample, which is a typical pattern of the α-helix (COCHRAN et al. 1952; FRASER and MACRAE 1973). Nine equatorial reflections were observed and indexed by a hexagonal unit cell of a = 1.680 nm (Table I). A meridional reflection corresponding to the unit height was observed at h = 0.1498 nm by tilting the oriented sample by 30° from the normal to the x-ray beam. Therefore, the molecular conformation is the standard α-helix with 18 residues per 5 turns in the repeating distance c = 2.696 nm. The density calculated for the unit cell containing one helix, 1.286 g cm^{-3}, agreed with the observed value.

In the c projection of this structure, the center of symmetry may be assumed. Then, the structure can be analyzed by means of Fourier synthesis if the signs of the structure factors are determined to be positive or negative. The cylindrically-averaged molecular structure factor on the equatorial layer line is given by the equation

$$F(R) = u\sum_j f_j J_o(2\pi R r_j)\exp(-B_j/4R^2) \qquad (1)$$

where R is the radial co-ordinate in reciprocal space, u = 18, f_j is the atomic scattering factor, r_j is the distance from the helix axis of the j-th atom in the residue, B_j is the temperature factor, and J_o is the zero-order Bessel function. The molecular structure factors were calculated for about twenty models with various side-chain conformations, which were built up by using standard bond lengths and bond angles (FRASER and MACRAE 1973; KOBELT and PAULUS 1974).

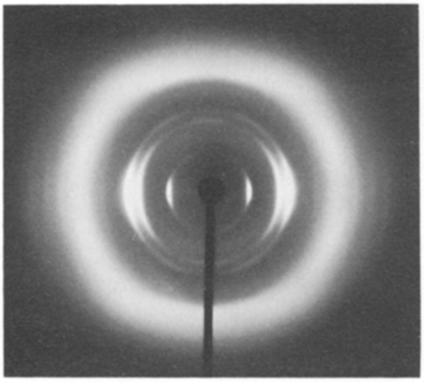


Fig. 2. X-ray pattern of the oriented PTLL film.

TABLE I

X-ray Data of PTLL

$hk\ell$	d_{obs}/nm	d_{calc}/nm	F
100	1.45	1.455	+176
110	0.840	0.840	+439
200	0.727	0.7275	+473
210	0.551	0.550	-54
300	0.485	0.485	-74
220	-	0.420	-
310	0.404	0.4035	-67
400	0.364	0.364	-103
320	0.334	0.334	-83
410	0.317	0.3175	-65

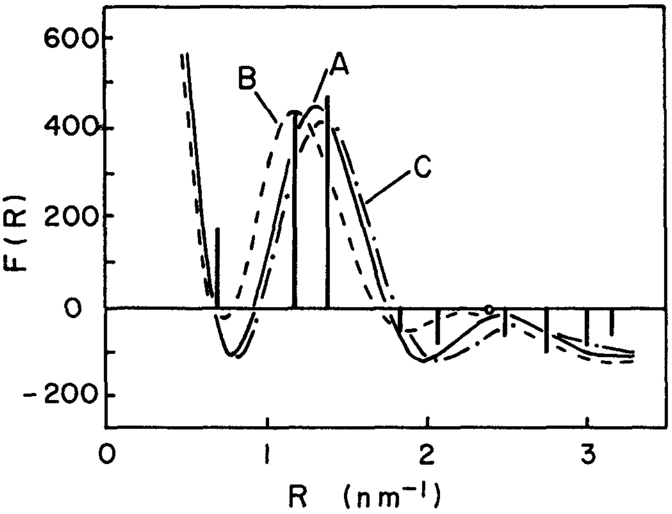


Fig. 3. Comparison between the observed structure factors (vertical rods) and the curves of *F(R)* calculated for models A, B, and C having the side-chain conformations illustrated in Fig. 4.

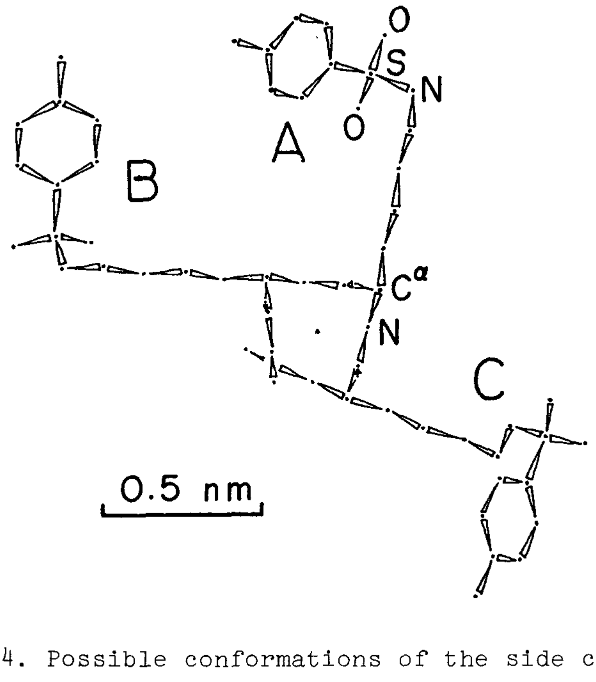


Fig. 4. Possible conformations of the side chain.

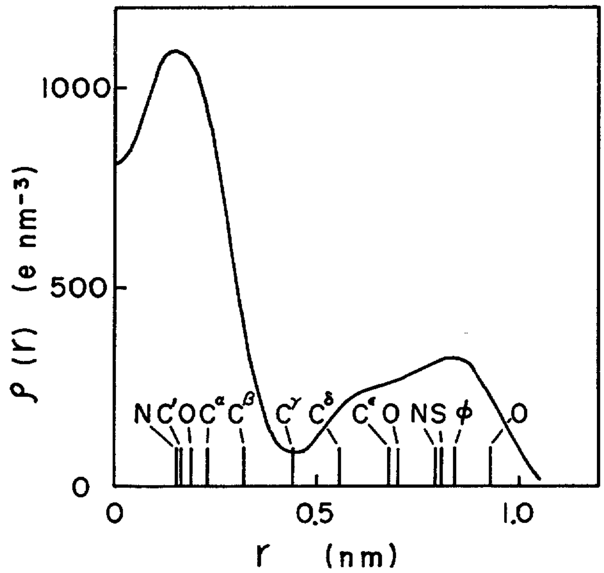


Fig. 5. Cylindrical radial electron-density distribution for an isolated single helix of PTLL.

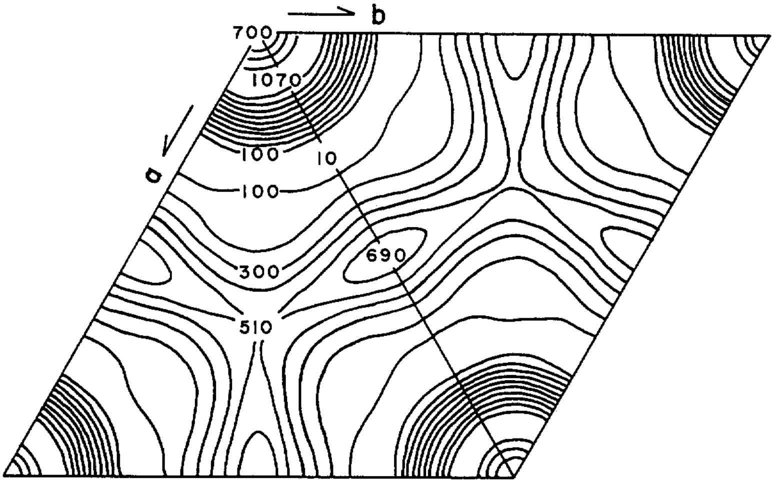


Fig. 6. Electron-density map of PTLL projected on the a b plane. Contours are at intervals of 100 e nm^{-3}.

Appropriate values of B_j were used by referring to the values in the case of poly(γ-benzyl glutamate) (MITSUI et al. 1967). These molecular transforms can be directly compared in absolute values with the structure factors of the equatorial reflections, since only one helix passes through the unit cell. Only a few models, in which *p*-toluenesulfonyl groups are located at $r \approx$ 0.8 nm, showed good agreements (Fig. 3). Possible models are illustrated in Fig. 4. The signs of the structure factors were thus determined, as given in the fourth column in Table I.

The structure factor $F(R)$ of an isolated single helix may be approximated by a smooth curve connecting the observed F values (vertical rods) in Fig. 3. The cylindrically-averaged radial electron-density distribution of an isolated single helix was numerically calculated by the equation

$$\rho(r) = \frac{1}{c}\int_0^C F(R)J_0(2\pi Rr)\,2\pi R\,\mathrm{d}R \tag{2}$$

and is shown in Fig. 5, where the atomic positions of model A are indicated for reference The electron-density map seen along the c axis was synthesized from the structure factors of all *hk0* reflections (Fig. 6).

The side-chain region in α-helical polypeptides having long side chains is generally disordered, and has been regarded as an amorphous microphase. In the present analysis, however, it is shown that the side chains are placed just at the intermediate position between the neighboring helices and therefore intervene mutually. These side-chain interactions may affect the physical properties.

The solid film of PTLL exhibited the thermal behavior like a glass transition (Fig. 7), which was considered to be associated with the onset of the side-chain motion. The transition temperature, estimated to be about 60°C, is much higher than 16°C for poly(γ-benzyl L-glutamate) and 8°C for poly(ε-carbobenzoxy L-lysine) (HIKICHI et al. 1975), both of which have rather

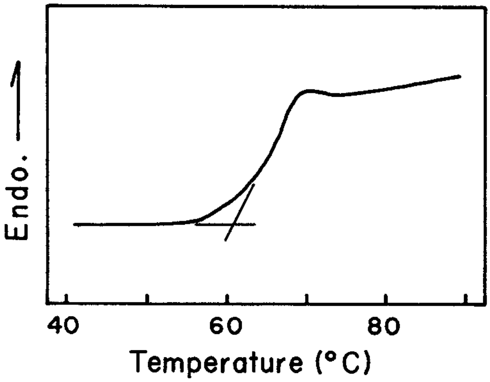


Fig. 7. DSC thermogram of the PTLL film.

shorter side chains than PTLL. This may be one of the effects of the side-chain interactions.

These side chains are expected to be regularly arrayed to some extent. Generally, solid films of polypeptides have not displayed the circular dichroism due to the side-chain chromophores, since the side chains are intrinsically achiral and those regions are disordered. However, thin films of PTLL exhibited a positive CD band at about 240 nm (Fig. 8). This induced CD suggests that some chromophores of *p*-toluenesulfonamide groups are coupled electronically in a helical array. The negative CD band at about 225 nm is due to the $n-\pi^*$ transition of the main-chain amide groups, indicating the right-handed helical sense. The peak height of the extrinsic CD band at 240 nm varied in a thermally reversible way (Fig. 9). A transition was also observed at about 60°C. These results indicate that the side chains in PTLL are arrayed in a dissymmetric manner and the cooperative motion takes place above 60°C. The dielectric properties of PTLL films will be reported elsewhere.

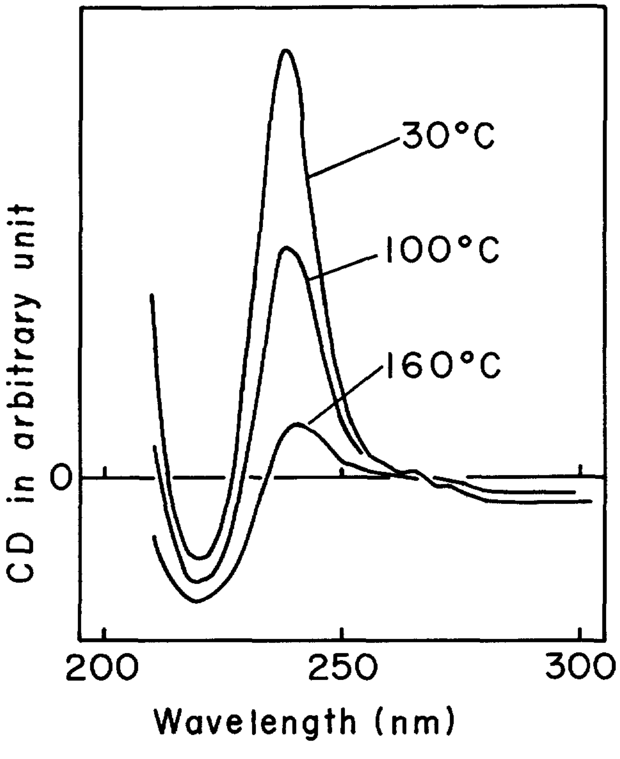


Fig. 8. CD spectra of PTLL films.

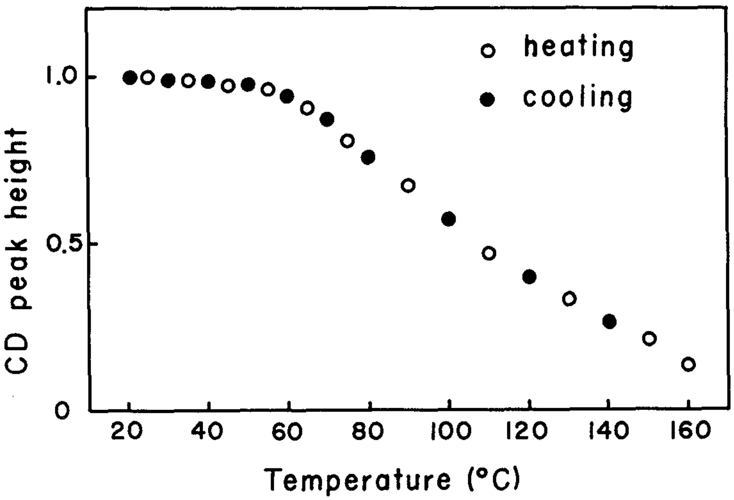


Fig. 9. Temperature dependence of the peak height of the positive CD band at about 240 nm.

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