

Side-Chain Relaxation Behavior of Racemic Mixtures of α -Helical Polypeptides Having Phenyl Groups at the End of the Side Chains

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SUMMARY

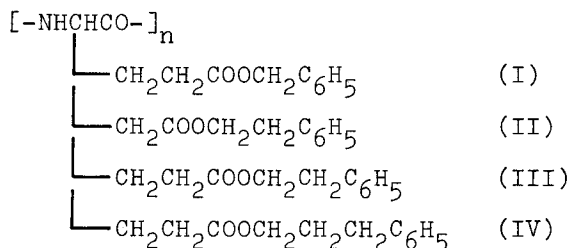
Side-chain interactions in racemic mixtures of α -helical polypeptides were investigated by means of dielectric measurements in relation to the regular stacks of phenyl groups in the racemic mixture of poly(γ -benzyl glutamate) (PBG). The samples used in this work were poly(β -phenethyl aspartate) (PPA), poly(γ -phenethyl glutamate) (PPG), and poly[γ -(3-phenylpropyl) glutamate] (PPPG). A dielectric dispersion due to the side-chain motion was observed around room temperature for all the samples. For PPA and PPPG, the dispersion shifted to lower frequencies by racemization, and the relaxation strength decreased in a similar manner as PBG. The racemic mixture of PPPG showed a thermally reversible transition at about 80°C, which was attributed to the breakdown and the formation of stacks of phenyl groups. Racemization had no effect for PPG. The conformational change from the right-handed α -helix to the left-handed π -helix which occurred at 140°C in the L isomer of PPA was not observed in the racemic mixture.

INTRODUCTION

Poly(γ -benzyl L-glutamate) (PBLG) exists as the right-handed α -helix (α_R) in solutions and in the solid state. The side-by-side molecular association and the large-scale fibrillar aggregation of PBLG in solutions have been considered to be due to the interaction of phenyl groups at the ends of the side chains. Several crystal modifications of PBLG (forms A to E), depending on the casting solvent, were different in the two-dimensional packing of the α -helices, i.e., in the manner of side-chain interactions (BAMFORD et al. 1956; MCKINNON and TOBOLSKY 1966 and 1968; WATANABE et al. 1981a; SASAKI et al. 1982). The phenyl-group association is very preferable in the racemic mixture of poly(γ -benzyl glutamate) (PBG), which is a mixture of equal amounts of PBLG and the D isomer. The regular stacks of phenyl groups in the racemic mixture of PBG

have been investigated by X-ray diffraction and other techniques (MITSUI et al. 1967; ELLIOTT et al. 1965; WATANABE et al. 1981b). Such a side-chain structure has considerable effects on the mechanical and dielectric relaxations which are due to the onset of the side chain motion (FUKUZAWA et al. 1974; TAKAHASHI et al. 1974). The racemic PBG exhibits a thermally reversible first-order transition at about 90°C, which is caused by the breakdown of regular stacks of phenyl groups (YOSHIKAWA et al. 1975; MATSUSHIMA et al. 1975).

In this work, the effects of racemization on side-chain interactions were investigated by means of dielectric measurements for α -helical polypeptides having similar side chains as PBG; poly(β -phenethyl aspartate) (PPA), poly(γ -phenethyl glutamate) (PPG), and poly[γ -(3-phenylpropyl) glutamate] (PPPG). Structural formulas of PBG (I), PPA (II), PPG (III), and PPPG (IV) are given as follows.



EXPERIMENTAL

The L and D isomers of PBG, PPA, PPG, and PPPG were prepared by polymerizing the corresponding N-carboxy-anhydrides in dioxane using triethylamine as the initiator. The molecular weights determined by viscosity measurements were about 150,000. Films of the L isomers and the racemic mixtures were prepared by casting the chloroform solutions onto glass plates at room temperature. The α -helical conformation in these films was confirmed by infrared absorption and X-ray diffraction techniques. Dielectric measurements were carried out in a three-terminal electrode system using conductive silver paste with an Ando Electric Co. TR-10C transformer bridge over a frequency range from 30 Hz to 1 MHz and a temperature range from 10 to 60°C. Differential scanning calorimetric (DSC) measurements were performed with a Perkin Elmer DSC Model II instrument.

RESULTS AND DISCUSSION

Solid-state structure

The crystal structures of the L isomer of PPA (PPLA) were discussed by some of the present authors in a

previous paper (SASAKI et al. 1981). The αR conformation of PPLA in the original film was transformed into the left-handed π -helix (πL) at about 140°C. The pleated-sheet β structure appeared at about 200°C. These forms and the structural transition were detected by DSC, infrared absorption, and X-ray diffraction measurements. The racemic PPA exhibited likewise two endothermic peaks at 145 and 195°C in the DSC measurements, which were irreversible. However, no structural change was found at the 145°C transition. The molecular conformation remained α -helical below 190°C, and it was transformed into the β structure above 200°C. The π -helical form could not be found in the racemic PPA. The side-chain interactions in the racemic PPA presumably make the π -helix unstable. The infrared frequencies of characteristic amide bands of PPA are listed in Table I.

The infrared spectra of the L isomers and the racemic mixtures of PPG and PPPG were essentially the same as those of α -helical PBLG reported by TSUBOI (1962). X-ray diffraction patterns showed that PPG and PPPG were highly crystalline, and that the crystal structures did not depend on the kind of the casting solvent. No difference was observed in the two-dimensional hexagonal lattice structures for the L isomer and the race-

Table I
Infrared Band Positions for Films of
Poly(β -phenethyl aspartate) [cm^{-1}]

polymer	form	amide	ester	amide	amide	amide
		A	C=O	I	II	V
PPLA	αR	3298	1742	1658	1553	602
	πL	3332	1731	1674	1536	604
	β	3276	1726	1634	1525	633
racemic mixture	α	3291	1738	1658	1554	607
	β	3276	1722	1631	1527	631

Table II
Two-dimensional Lattice Parameters

polymer	L form	racemic form
PBG	(form C type) $a = b = 14.95\text{A}$ $\gamma = 121^\circ$	hexagonal $a = b = 14.9\text{A}$
PPA	(αR form) disordered	tetragonal $a = b = 13.6\text{A}$
PPG	hexagonal $a = b = 15.6\text{A}$	hexagonal $a = b = 15.6\text{A}$
PPPG	hexagonal $a = b = 16.3\text{A}$	hexagonal $a = b = 16.3\text{A}$

mic mixture of both PPG and PPPG. The lattice parameters are summarized in Table II.

The DSC thermogram of the racemic mixture of PBG is shown in Fig. 1, exhibiting the same feature as previously reported. The thermally reversible transition has been attributed to the breakdown and the formation of regular stacks of phenyl groups. The racemic PPPG exhibits a broad transition at about 80°C (Fig. 1), which may be also attributed to the same origin as in the case of PBG. The racemic PPG did not show any transition.

Dielectric properties

From the above-mentioned results, the racemization effect was observed for PPA and PPPG, but not for PPG. If strong side-chain interactions are formed in racemic mixtures, the side-chain dispersion may become broader and shift to lower frequencies (i.e., to higher temperature), and the relaxation strength may decrease to some extent owing to the restricted side-chain motion. These features have been observed for PBG (TAKAHASHI et al. 1974).

Fig. 2 compares the frequency dependence of the dielectric constant (ϵ') and the loss factor (ϵ'') of

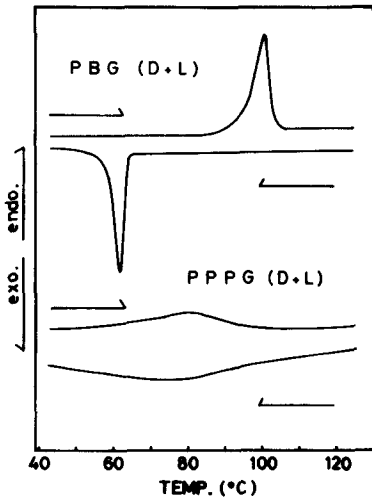


Fig. 1. DSC thermograms of the racemic mixtures of PBG and PPPG

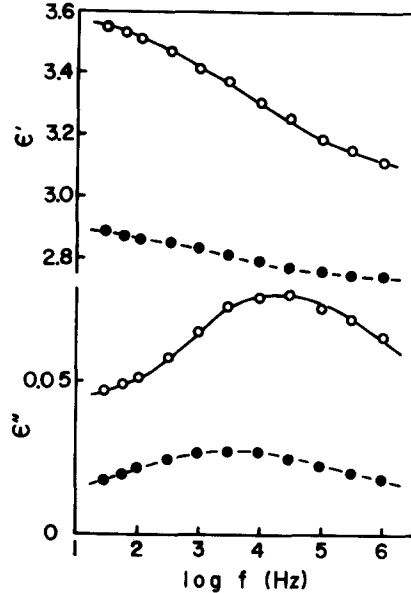


Fig. 2. Frequency dependence of ϵ' and ϵ'' of PPLA (o) and the racemic PPA (●) at 40°C

PPLA and the racemic PPA at 40°C. The feature is just like that of PBG, and suggests that the side-chain motion in the racemic PPA is restricted to a great extent. If phenyl groups are regularly stacked, a transition like those of PBG and PPPG (Fig. 1) may be predicted. No thermal transition, however, took place for the once-annealed sample of the racemic PPA. Although the side-chain motion is sterically hindered, the phenyl groups may not be regularly stacked. These dielectric properties indicate directly that the situation of the side-chain interactions in the racemic PPA is quite different from that in PPLA. It may be one reason for missing π -helical form in the racemic PPA.

Fig. 3 shows the frequency dependence of ϵ' and ϵ'' of PPG and PPPG at 30°C. Racemization has no effect for PPG, while PPPG exhibits the behavior similar to that of PBG or PPA. As inferred from the thermal behavior (Fig. 1), the side chains in the racemic PPPG may be partly in the stacked state, which is probably not so regular as PBG.

In Fig. 4, the logarithms of frequencies at loss maxima are plotted against reciprocal temperatures for the L isomers and the racemic mixtures of PPA, PPG, and PPPG. The relationship between the relaxation time and temperature is of an Arrhenius type. Apparent activation energies were 30–35 kcal/mol.

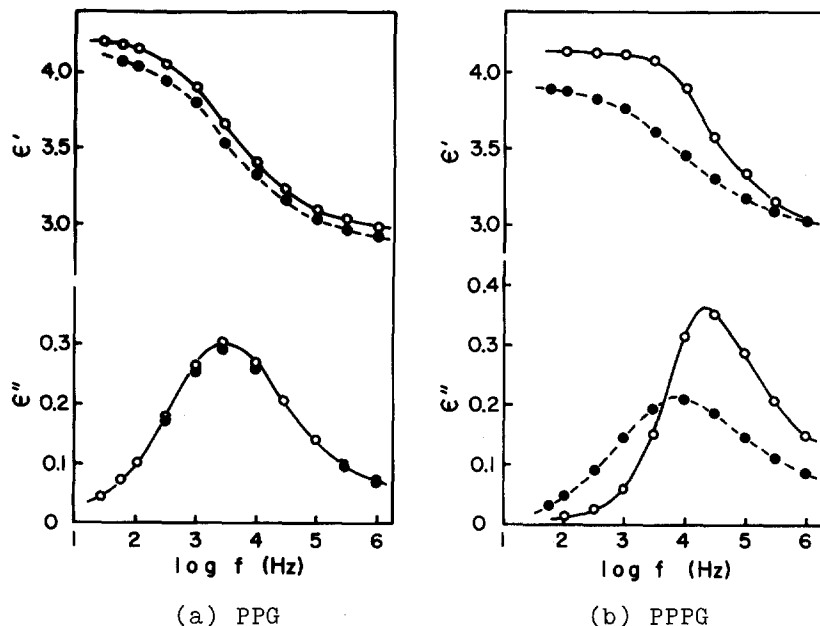


Fig. 3. Frequency dependence of ϵ' and ϵ'' of PPG and PPPG at 30°C. (o) L isomer, (●) racemic mixture

If the side chain is sufficiently long, the surface chirality of the α -helical molecule will be diminished so that the racemization may not produce an appreciable effect. The present results show that the chirality is preserved even in PPPG. No effect of racemization for PPG suggests that the side-chain conformation is such that the chirality is not effective. The side-chain region in PPG may be disordered so that the recognition of adjacent L and D molecules is impossible

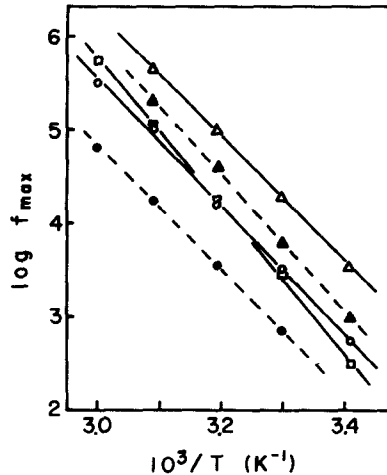


Fig. 4. Plots of $\log f(\max)$ against reciprocal temperature for PPLA (o), the racemic PPA (●), PPLG and the racemic PPG (□), PPLG (Δ), and the racemic PPPG (▲)

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