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Molecular Conformations of D,L-Alternating Polypeptides

1. Poly(γ-Benzyl Glutamate) and Poly(γ-Phenethyl Glutamate)

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SUMMARY

D,L-Alternating poly(γ-benzyl glutamate) (PBDLG) and poly(γ-phenethyl glutamate) (PPDLG) were prepared by the racemization-free method proposed by Caille et al. (1) for the synthesis of PBDLG. Both polymers were fractionated into two components, (A) and (B), using solubility differences in DMF, methanol, and ethanol. Infrared and X-ray data support the α-helical molecular conformation for the (A) films obtained from chloroform solutions, as was reported by Heitz et al. (2) for PBDLG. The molecular conformation of PPDLG-(B) is essentially the same as that of PBDLG-(B), which was assigned to the antiparallel-chain polar pleated-sheet structure by Lotz et al. (3). The ω-helical conformation was found for PBDLG by exposing the (A) film to the vapor from DMF with a small amount of water, but not for PPDLG.

INTRODUCTION

The helical conformations of poly(α-amino acid)s, [-NH-CHR-C'O-]$_n$ (R: side chain), are stabilized by the intramolecular hydrogen bonds between NH and C'O groups (4,5). The helices are described by the notation n_r, where n denotes the number of residues per turn and r the number of atoms in the ring formed by a hydrogen bond (6). The right-handed α (3.6_{13}) helix has been generally observed for L polypeptides. The existence of the 3_{10} and 5.1_{17} (γ) helices has not yet been established (7). The conformational versatility of polyaspartic esters (R = $-CH_2COOR'$) has been extensively investigated. Poly(β-benzyl L-aspartate) (R' = $-CH_2C_6H_5$), for instance, can exist as the right- and left-handed α-helices and the left-handed ω (4_{13}) helix (8). The left-handed π (4.25_{16}) helix was proposed for poly(β-phenethyl L-aspartate) (R' = $-CH_2CH_2C_6H_5$) (9).

For the D,L-alternating polypeptides, Ramachandran et al. (10) suggested possible existence of some additional species other than the 3_{10}, 3.6_{13} (α), and 4.4_{16} (π) helices from the conformational energy calculations. In the LD_3 helix of Ramachandran et al. (the π_{DL} helix denoted by Spach et al.), the D and L residues assume different orientations with alternating two kinds of hydrogen bonds of 14 and 16 atoms in the rings (2).

Caille et al. (1) prepared D,L-alternating poly(γ-benzyl glutamate) (PBDLG) (R = $-CH_2CH_2COOCH_2C_6H_5$) and separated this polymer into two components, (A) (insoluble in DMF) and (B) (soluble in DMF). They reported that PBDLG-(A) adopts the α-helical conformation in the film obtained from chloroform solution, and that the α form was transformed into the π_{DL} form at 130-150°C and furthermore into the double-stranded helix at 220-230°C (11,12). For the PBDLG-(B) film, Heitz et al. (2,13) assumed the antiparallel-chain polar pleated-sheet structure (Figure 1) proposed by De Santis et al. (14), where the direction of the polypeptide chain is perpendicular to the orientational direction just like the so-called cross-β type but all the carbonyl groups are oriented in a polar way. However, their sample of the (B) component, which was obtained by extracting for 10 days with hot ethanol, was soluble in helicogenic solvents such as DMF and *m*-cresol (1,3). The molecular weight was quite low.

The side-chain group has considerable effects on the main-chain conformation. We synthesized D,L-alternating poly(γ-phenethyl glutamate) (PPDLG) (R = $-CH_2CH_2COOCH_2CH_2C_6H_5$) and investigated the molecular conformation in comparison with that of PBDLG.

EXPERIMENTAL

PBDLG and PPDLG were prepared according to the racemization-free method proposed by Caille et al. (1) for the synthesis of PBDLG. In their process, the amino group was protected by using 2-nitrophenylsulphenyl group and also *tert.*-butoxycarbonyl (Boc) group. For the synthesis of PPDLG in this work, the protection by Boc group proved to be successful.

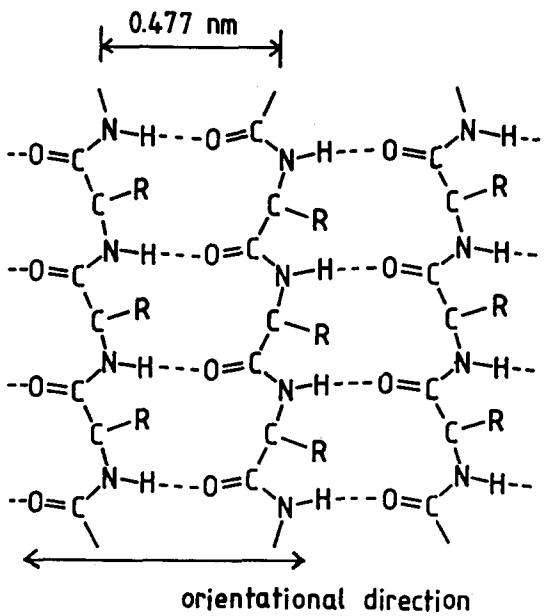

Figure 1. Polar pleated-sheet structure (14).

The polycondensations were carried out in benzene by using tetrapeptide 2-hydroxyphenyl esters. Crude samples of PBDLG and PPDLG were obtained from the reaction mixtures by precipitating with ethanol.

The crude sample of PBDLG contained a rather high molecular weight fraction, which was almost insoluble in DMF and isolated as (A) component. The residue obtained from the filtrate involved another portion (termed (B) component), in addition to a small amount of (A) component which was removed by reprecipitating from dichloroacetic acid (DCA) with methanol. The (B) component was recovered from the DCA solution by precipitating with water.

The sample of PPDLG was first separated into two portions, insoluble in DMF (termed (B_1) component) and soluble in DMF (a mixture of (A) and (B_2) components). The PPDLG-(A) component was precipitated from the DCA solution with ethanol. The (B_2) component was recovered from the DCA solution by precipitating with water in the same way as PBDLG-(B). The (B_1) component might be formed in the polymerization process, and the molecular weight is probably higher than that of (B_2) component, since it was insoluble in DMF. The molecular weight of PPDLG-(A) might be higher than that of (B_1) component, but lower than that of PBDLG-(A), since PPDLG-(A) was dissolved in DMF.

Solid films were prepared from these fractions by drying the chloroform solutions over glass plates, although the solubility of PPDLG-(B_1) in chloroform was rather poor. Oriented specimens were obtained by stroking chloroform solutions onto glass plates with a spatula along one direction until the solvents had evaporated.

RESULTS AND DISCUSSION

Infrared frequencies of characteristic amide bands ob-

TABLE I

Infrared Band Positions for Films of PBDLG and PPDLG [a]

Polymer	Fraction	Band Position, cm^{-1}			References
		Amide A	Amide I	Amide II	
PBDLG	(A)	3290	1664	1550	(2)
	(B)	(3290)	1690(sh) 1629	1525	(1)
PBDLG	(A)	3280	1661	1548	this work
	(B)	3270	1690(sh) 1627	1531	
PPDLG	(A)	3280	1661	1547	this work
	(B_1)	3276	1687(sh) 1621	1536	
	(B_2)	3272	1690(sh) 1626	1530	

[a] All the films were prepared from the chloroform solutions. Shoulder bands are indicated by (sh).

served for PBDLG and PPDLG are listed in Table I. The spectrum of PBDLG-(A) is given in Figure 2. Our specimen of PBDLG-(A) was identified to be the same as that of Heitz et al. (2), and assigned to the α-helical form. The feature of the infrared spectrum of PBDLG-(B) (this work) was consistent with the data by Cailleet al. (1), except for the amide A band. The amide frequencies of PPDLG-(A) coincided with those of PBDLG-(A). The frequencies observed for PPDLG-(B_1) and (B_2) closely resembled those for PBDLG-(B).

The PBDLG-(A) oriented film showed an X-ray diffraction pattern characteristic of the α-helical form. Two meridional reflections were observed at spacings 0.148 and 0.297 nm. The 0.148-nm spot corresponds to the unit height, h, while the 0.297-nm one to the height per dipeptide unit. The equatorial reflections were explained by a hexagonal unit cell of a = 1.49 nm, while Heitz et al. (2) reported the dimension a = 1.53 ± 0.01 nm. The density calculated from the values a = 1.49 nm and h = 0.148 nm is 1.28 g cm^{-3} in agreement with the observed value (1.25 g cm^{-3}).

The PBDLG-(B) oriented film exhibited the X-ray pattern with a strong meridional reflection of 0.473 nm and its second, third, and fourth order reflections. From the periodicity of 0.473 nm along the orientational direction, Heitz et al. (2,13) interpreted the structure by the antiparallel-chain polar pleated-sheet type (Figure 1). They mentioned that the equatorial line in the X-ray pattern of the PBDLG-(B) film prepared from the *m*-cresol solution comprised a 2.1-nm streak and many arcs (3). The equatorial Bragg reflections observed for our sample were explained by a hexagonal lattice of side a = 2.29 nm. The two-dimensional arrangement of the polar pleated-sheets is not clear. The structure of PBDLG-(B) may need more detailed investigation.

The PPDLG-(A) oriented film showed the X-ray pattern with

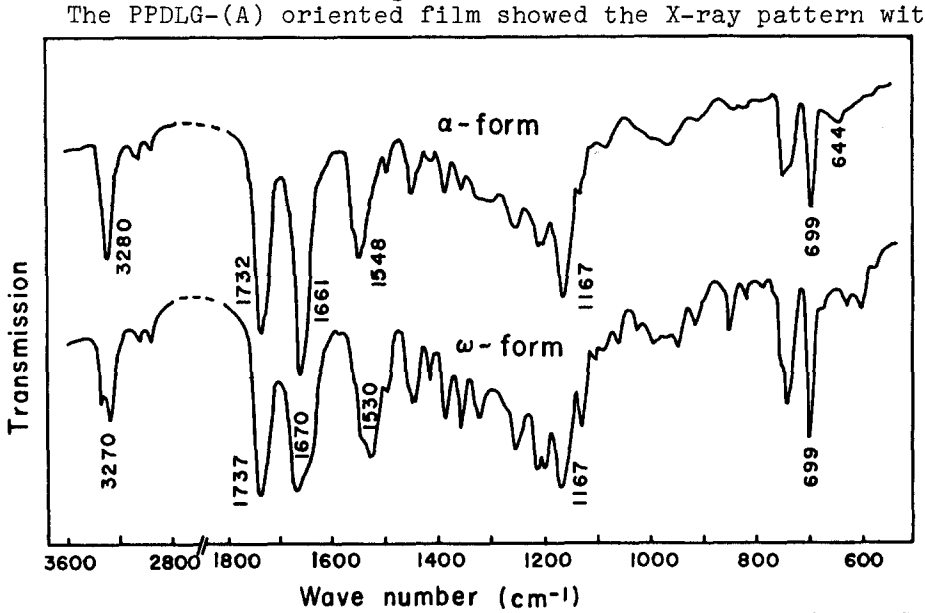

Figure 2. Infrared spectra of the α and ω forms of PBDLG.

the meridional reflections of 0.147 and 0.294 nm, which was similar to that of PBDLG-(A). The equatorial reflections were explained by a hexagonal lattice of a = 1.59 nm. The calculated density, 1.20 g cm^{-3}, agreed with the observed value.

The X-ray patterns of PPDLG-(B_1) and (B_2) films were similar to that of PBDLG-(B). The layer-line spacings of (B_2) were elucidated by the 0.475-nm periodicity in the same way as PBDLG-(B), while the equatorial ones by a hexagonal lattice of a = 2.42 nm. Spacings of major layer lines of PPDLG-(B_1) were explained by the 0.478-nm periodicity, but a few weak layer lines elucidated by the doubled spacing 0.956 nm were also observed. As inferred from the infrared data, the molecular conformations of (B_1) and (B_2) may be essentially the same. Broad equatorial reflections of (B_1) were observed at 2.2, 1.15, and 0.75 nm, suggesting that the distance between neighboring pleated sheets is about 2.2 nm. This indicates that the structure is similar to that proposed for poly(D-alanyl-L-alanyl-D-valyl-L-alanine) by Heitz et al. (13).

From the infrared and electron diffraction studies, Heitz et al. (2) predicted that the PBDLG might assume the ω-helical (4_{13}) conformation in the film prepared from the solution in DMF containing a small amount of water. In this work, the oriented ω form of PBDLG could be prepared by exposing the PBDLG-(A) film to the vapor from DMF + 10 vol% water and subsequently drying in vacuo. The infrared spectrum is given in Figure 2. In the amide A region, a small absorption was observed at 3335 cm^{-1}. This may suggest that the NH groups are partly in the free state. The amide I and II frequencies (1670 and 1530 cm^{-1}, respectively) are consistent with those (1675 and 1536 cm^{-1}) observed for the ω form of poly(β-benzyl L-aspartate) (15).

The X-ray pattern of the oriented ω form of PBDLG and its reciprocal-space rotation diagram are shown in Figure 3. Nineteen equatorial reflections and twenty-one higher-layer-line ones were explained by an orthorhombic unit cell with dimensions a = 2.692 nm, b = 1.554 nm, and c(chain axis) = 0.540 nm. A meridional reflection of 0.135 nm was observed on the fourth layer line, corresponding to the unit height. From a simple consideration on the equatorial intensity distribution, two helices pass through the unit cell. The arrangement of the helical axes is exactly hexagonal. In the crystal lattice, four kinds of helices (with combinations of up- or down-pointing and right- or left-handed helices) may be packed with some extent of positional order. The observed density, 1.26 g cm^{-3}, was consistent with the calculated value, 1.29 g cm^{-3}.

In the ω form of poly(β-benzyl L-aspartate), the left-handed fourfold helices are packed in the tetragonal array with a = 1.388 nm. The ω-helix of the D,L-alternating polypeptide has no longer the fourfold helical orientation of the side chains; the symmetry is reduced to the twofold helix. This molecular symmetry may be responsible for the two-chain orthorhombic unit cell.

We could prepare the ω form of PBDLG also by the very slow evaporation of the solvent from the chloroform solution. This fact may indicate that the α-helix is stable in the solution, while the ω-helix is preferable in the solid state.

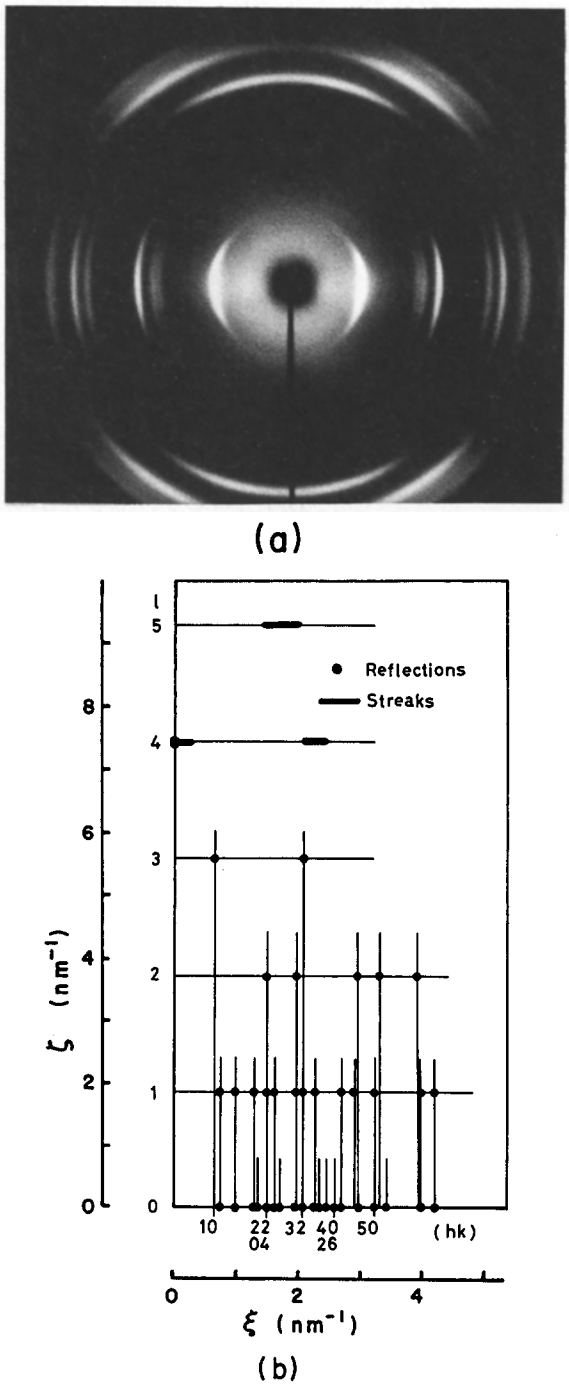

Figure 3. (a) Flat-plate X-ray diffraction photograph and (b) its reciprocal-lattice rotation diagram of the ω form of PBDLG.

On the other hand, the α form of PPDLG was not transformed into the ω form but into the (B_2) form by treating with the DMF + water vapor. Various attempts to prepare the ω form of PPDLG had failed. In a previous paper (16), we discussed the structures and properties of poly(γ-benzyl glutamate) (PBG) and poly(γ-phenethyl glutamate) (PPG). In the solid state, the phenyl groups of PBG can stack to each other, but those of PPG do not. The difference in the side-chain length has some considerable effects, and may affect the stability of the ω form of the present D,L-alternating polymers.

The α form of PBDLG-(A) was transformed into another conformation by annealing at about 140°C, which was assigned to the π_{DL} form by Heitz et al. (2). Similar transition was observed for PPDLG. Conformational changes caused by annealing will be discussed in the following paper.

REFERENCES

1. A. Caille, F. Heitz, and G. Spach, J. Chem. Soc. Perkin I. 1621 (1974).
2. F. Heitz, B. Lotz, and G. Spach, J. Mol. Biol. 92, 1 (1975).
3. B. Lotz, F. Heitz, and G. Spach, C. R. Hebd. Seances Acad. Sci. Ser. C 276, 1715 (1973).
4. L. Pauling, R. B. Corey, and H. R. Branson, Proc. Natl. Acad. Sci. U. S. A. 37, 205 (1951).
5. B. W. Low and H. J. Grenvelle-Wells, Proc. Natl. Acad. Sci. U. S. A. 39, 785 (1953).
6. IUPAC-IUB Commission on Biochemical Nomenclature, J. Mol. Biol. 52, 1 (1970).
7. B. V. V. Prasad and V. Sasisekharan, Macromolecules 12, 1107 (1979).
8. E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, and W. E. Hanby, J. Mol. Biol. 5, 230 (1962).
9. S. Sasaki, Y. Yasumoto, and I. Uematsu, Macromolecules 14, 1797 (1981).
10. G. N. Ramachandran and R. Chandrasekaran, Ind. J. Biochem. Biophys. 9, 1 (1972).
11. F. Heitz, B. Lotz, and G. Spach, C. R. Hebd. Seances Acad. Sci. Ser. C 280, 1509 (1975).
12. B. Lotz, F. Colonna-Cesari, F. Heitz, and G. Spach, J. Mol. Biol. 106, 915 (1976).
13. F. Heitz, G. Detriche, F. Vovelle, and G. Spach, Macromolecules 14, 47 (1981).
14. P. De Santis, S. Morosetti, and R. Rizzo, Macromolecules 7, 52 (1974).
15. E. M. Bradbury, B. G. Carpenter, and R. M. Stephens, Biopolymers 6, 905 (1968).
16. M. Nagao, S. Sasaki, T. Hayashi, and I. Uematsu, Polym. Bulletin 9, 11 (1983).

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