

Conformation Studies of Copolypeptides Composed of γ -Methyl-D-glutamate and γ -Methyl-L-glutamate

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ABSTRACT: Copolypeptides composed of γ -methyl-D-glutamate and γ -methyl-L-glutamate covering the whole range of copolymer compositions were synthesized by the NCA method using triethylamine (TEA) as the initiator. The X-ray diffraction measurements for these D, L-copoly(γ -methyl glutamate)s in solid state indicated that these copolypeptide molecules can exist in a conformation very similar to that of the α -helix of PMLG or PMDG. Infrared absorption spectra of solid films were also measured. The sum of right- and left-handed helix contents was determined from intensities of amide V bands. Optical rotatory dispersions of these copolymers in helicogenic solvents were used to ascertain their helical character. The parameter b_0 of Moffitt—Yangs equation for D, L-copoly(γ -methyl glutamate) in helicogenic solvent could give the difference between right- and left-handed helix content. Thus the right-handed helix, left-handed helix, and random coil contents were estimated separately. In order to test the relative stability of helices of these copolypeptides, the optical rotatory dispersions were examined in DCA—DCE mixtures as functions of solvent composition.

KEY WORDS Polypeptide / Copolymer of γ -Methyl-D-glutamate and γ -Methyl-L-glutamate [D, L-copoly(γ -methyl glutamate)] / Chain Conformation / X-ray Diffraction / IR / Viscosity / Optical Rotatory dispersion / Helix Stability /

The conformation of synthetic homopolypeptides has been investigated in many respects. However, with respect to copolypeptides composed of D- and L-enantiomorph amino acids a number of problems still remain to be solved. Primarily, the conformation should be discussed in connection with the chain configuration governed by the polymerization mechanism. In this sense, conditions of polymerization, such as the kind of initiator and solvent, and the polymerization field, may be of major importance for chain configuration represented, for example, by the distribution of optical isomers along the chain. Lundberg and Doty¹, performed for the first time, a conformation study on D, L-copoly(γ -benzy glutamate) (PBDLG) polymerized from D- and L-N-carboxy anhydrides by using *n*-hexylamine as the initiator and dioxane as the solvent. It was concluded that the chain configuration of PBDLG thus prepared is rather stereoblock, *i.e.*, the sequential probability that

a D-residue (or L-residue) is succeeded by the same kind of residue is considerably larger than 0.5. Wada², and Tsuboi, *et al.*³, introduced optical rotatory dispersion and infrared spectra measurements to determine the helix and coil contents, and estimated these contents for PBDLG. According to Wada², helices characterized by a unique helical sense are maintained even for D, L-copolymer containing up to about 25-% minor component. This means that minor D-residues(L-residues) may be incorporated in right-handed(left-handed) helix mainly composed of major L-residue(D-residues).

More recently, Masuda, *et al.*,⁴ published results on D, L-copoly(γ -methyl glutamate) (PMDLG), polymerized with sodium methoxide as the initiator and dioxane as the solvent, and pointed out that the formation of the left-handed helix is appreciable even at low D-residue content.

In the present study, PMDLG covering the whole range of D, L compositions was synthesized

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using triethylamine (TEA) as the initiator, and dioxane—methylene dichloride-(1:1) mixture as the solvent. The infrared spectra and X-ray diffraction patterns of solid films were analyzed. Furthermore the helix content was estimated by optical rotatory dispersion measurements with copolypeptides dissolved in heliogenic solvents. The relative stability of helices of these copolypeptides were also investigated from the optical rotatory dispersions in DCA—DCE mixture.

EXPERIMENTAL

Synthesis of Copolypeptides and Estimation of Molecular Weights

The *N*-carboxy anhydrides of γ -methyl-D- and L-glutamate, respectively, were prepared by reacting γ -methyl-D- and L-glutamate monomers with phosgene, according to the method proposed by Blout⁵. Both D-monomer NCA and L-monomer NCA were recrystallized several times from ethyl acetate with petroleum ether. D- and L-NCA were mixed at various ratios and were polymerized in 1:1 (v/v) mixture of dry dioxane and methylene dichloride. The total anhydride concentration was kept at 2%. The polymerization initiator used here was TEA. The anhydride-to-initiator ratio was varied, hence the

copolypeptide obtained were of different degrees of polymerization. Polymerization was completed within about 72 hr at 25°C. When the polymerization reached about 70–80%, the reaction mixture was poured into a large amount of cold methanol. The precipitated material was dried under reduced pressure at 50°C.

The intrinsic viscosity $[\eta]$, as a measure of the molecular size of these copolypeptides, were determined in dichloroacetic acid (DCA) at 25°C with Ubbelohde-type viscometers.

The results of the polymerization are summarized in Table I, in which series A and B samples, respectively, were prepared with anhydride-to-initiator ratios of 500 and 200 (50 for B-12).

Molecular weight determination of some of the PMDLG samples was performed using a Mechrolab Model 502 High Speed Membrane Osmometer. The solvent used for the measurements was *m*-cresol, and the temperature was fixed at 50°C.

Infrared Spectra

Infrared absorption spectra of solid films of the copolypeptides cast from the dilute solutions were measured with a Perkin-Elmer Model 521 spectrophotometer in a region of 4000–350 cm⁻¹. The solvents used for preparing the solid

Table I. Samples of D, L-copoly(γ -methyl glutamate)

Sample No.	[M]/[I], mol/mol	Monomer ratio, L%	Polymer composition, L%	Conversion %	$[\eta]$ (DCA, 25°C), dl/g	P_n
A-1	500	100.0	100.0	79	2.78	1700
A-2	500	90.0	90.9	81	1.38	650
A-3	500	80.0	80.6	81	1.08	520
A-4	500	69.9	69.3	80	0.97	
A-5	500	60.0	60.1	77	0.88	
A-6	500	50.0	50.6	72	0.91	
A-7	500	40.0	39.8	75	0.97	
A-8	500	30.0	29.5	77	1.14	
A-9	500	20.0	19.0	78	1.39	
A-10	500	10.0	9.0	80	1.81	
A-11	500	0.0	0.0	82	3.45	
B-12	50	100.0		81	0.97	450
B-13	200	90.0		69	1.26	
B-14	200	80.0		70	0.72	
B-15	200	70.0		66	0.52	
B-16	200	60.0		67	0.53	
B-17	200	50.0		65	0.59	

films were chloroform-trifluoroacetic acid(TFA)-(95 : 5 v/v) mixture (a helicogenic solvent), and TFA (a random coil solvent), these being chosen to study the effect of the type of solvent from which films were cast.

X-ray Diffraction

X-ray diffraction photographs were obtained with oriented solid films of a PMDLG, PMDG, PMLG, and a mixture of PMDG and PMLG. All of these films were prepared by casting from TFA solutions and stretched about 100% in one direction in water at 45°C. X-ray photographs were taken with a flat-plate camera by using nickel-filtered Cu-K α radiation generated from a Rigaku-Denki Type D-3F X-ray generator.

Optical Rotatory Dispersion

Optical rotatory dispersions in the range 15 to 25°C were measured with a Yanagimoto OR-100-type spectropolarimeter using a tungsten lamp as the light source. The wavelength used ranged from 325 to 610 m μ . The concentration of each copolymer solution was 1.0 g/dl throughout these measurements. The solvent systems used were DCA—DCE mixtures and *m*-cresol. The polymer composition represented by *L*% was determined from the effective residue rotation, $[m']$, for copolymers dissolved in DCA, a random coil solvent,

$$L\% = 100 \frac{[m'] - [m']_D}{[m']_L - [m']_D} \quad (1)$$

where subscript D and L represent corresponding homopolypeptides.

RESULTS AND DISCUSSION

Polymer Composition and Molecular Weight Determination

Table I show the samples used for the experiment. The polymer composition represented by *L*%, shown in the forth column of Table I, was determined by the method mentioned above. It should be noted that the *L* percentages in copolypeptides are slightly different from those referred to the amount of NCA's used for polymerization.

Number-average degrees of polymerization P_n were determined for some of the copolypeptides, and the results obtained are shown in the last

column of Table I.

X-ray Diffraction Measurements

Figure 1 shows some of the X-ray diffraction photographs of PMLG, PMDG, PM(D+L)G- (equimolar blend of PMDG and PMLG), and PMDLG(equimolar D,L-copoly(γ -methyl glutamate), sample A-6). In Figure 1, the X-ray beam was aligned parallel to the surface of sample films and the direction of stretching in the films was perpendicular to the X-ray beam. The photographs obtained indicate that PMLG, PMDG, and PM(D+L)G films are more oriented than PMDLG film.

According to Bamford, Hanby, and Happey^{6,7}, the unit cell of PMLG is hexagonal with $a=b=11.95 \text{ \AA}$ and $c(\text{fiber axis})=27.0 \text{ \AA}$. The observed and calculated spacings are given in Table II, together with the relative intensities of the reflections. Calculation of spacings assuming a hexagonal unit cell was carried out by Bamford, *et al.*⁶ With respect to PMLG and PMDG, all of these measured reflections were almost equal to calculated value. On the other hand, the relative intensities of the reflections for PMDLG film (Sample A-6) were much weaker than those for PMLG or PMDG except that of the (100) reflection. However the locations of reflections observed with PMDLG are very similar to these of PMDG or PMLG. These observations may indicate that PMDLG film consists of fewer crystalline portions and that these crystalline portions are almost the same as those of PMLG or PMDG in crystalline system. Wada, *et al.*,³ studied PBDLG with various D/L ratios. From infrared absorption and X-ray diffraction measurements, they concluded that each chain of those copolymers consists of random coil and helix portions and that the conformation of the later portion is similar to that of the α -helix of pure PBLG.

The X-ray diagram (Figure 1c) of the 50—50% mixture of PMDG and PMLG, *i.e.*, PM(D+L)G, differs from that (Figure 1) of equimolar PMDLG, If, in the process of copolymerization, the selectivity of D- or L-residue is extremely high, then a 50—50% mixture of L- and D-polypeptide would result from the polymerization of a 50—50% mixture of the NCA's of L- and D-amino acids. The fact that the X-ray diffraction

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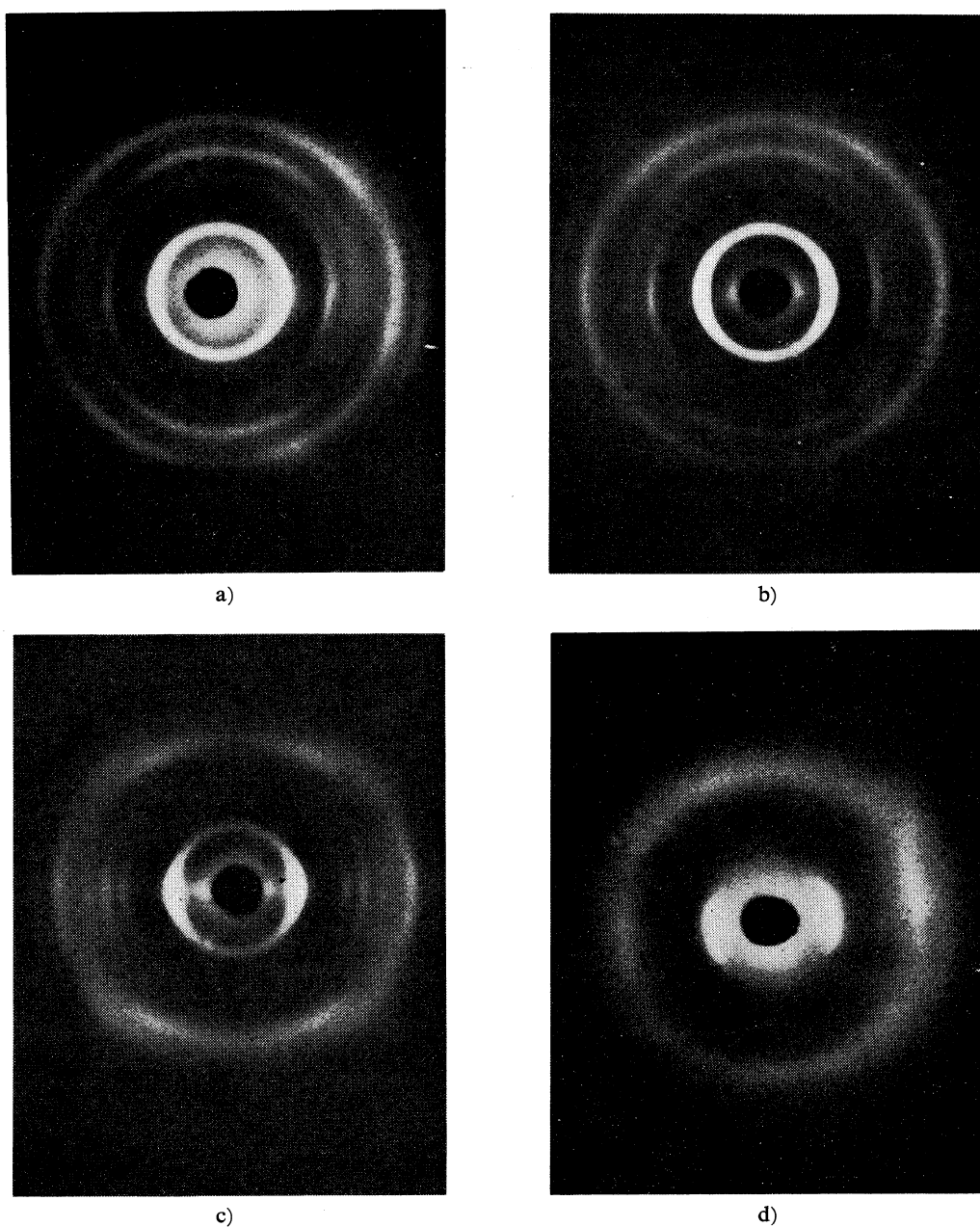


Figure 1. X-ray diffraction photographs of (a) PMLG [sample A-1], (b) PMDG [sample A-11], (c) PM(D+L)G [an equimolar blend of sample A-1 and sample A-11], and (d) PMDLG ($L=50$ mol%) [sample A-6]. $\text{Cu-K}\alpha$ radiation. The X-ray beam was irradiated parallel to the surface of the specimen films and the direction of stretching in the films was the vertical direction in the photographs.

Table II. X-ray diffraction of oriented solid films of PMLG, PMDG, PM(D+L)G blend, and PMDLG(L=50 mol%)^a

Index	$d(\text{calcd}), \text{\AA}$	$d(\text{obsd}), \text{\AA}$			
		A-1 (PMLG)	A-11 (PMDG)	A-1/A-11 [PM(D+L)G]	A-6 (PMDLG)
(100)	10.35 vs	10.34 vs	10.31 vs	10.31 vs	10.36 vs
(110)	5.94 s	5.99 s	5.98 s	5.99 s	6.02 w
(200)	5.18 w	5.20 w	5.25 w	5.23 s	5.24 vw
(210)	3.91 s	3.90 s	3.87 s	3.86 s	—
(300)	3.45 m	3.44 s	3.43 s	3.43 m	3.48 vw
(105)	4.79 s	4.85 s	4.82 s	4.44 s	4.80 w
(115)	4.01 s	4.03 s	4.06 s	3.76 m	4.04 vw
(108)	3.21 vw	3.16 vw	3.23 w	3.28 vw	3.27 vw
(208)	2.83 w	2.83 w	2.85 w	—	—

^a Hexagonal unit cell; $a=b=11.95 \text{\AA}$; $c=27.0 \text{\AA}$.

patterns obtained with PM(D+L)G and PMDLG are different each other may suggest that polymerization of the present system proceeds in such a way as to yield rather randomly sequenced copolymers.

Infrared Absorption Measurements

Figure 2 illustrates the infrared spectra measured with unoriented films of PMDLG having different copolymer compositions. The aliphatic C—H stretching vibration gives rise to the band at 2950 cm^{-1} and the absorption coefficient of this vibration is expected to be independent of the chain conformations. However the amide V band, assigned to the N—H out-of-plane bending, has been found^{8,9} to be sensitive to the chain conformations even for unoriented samples. According to the study of Miyazawa, *et al.*⁸, the amide V band of the α -helix appeared at $610\text{--}620 \text{ cm}^{-1}$ for PMLG, PBLG, and poly(L-alanine), while that of the disordered form was at 650 cm^{-1} , without being affected by the kind of side chains.

In Figure 2, the amide V band of the α -helix was observed at 615 cm^{-1} for PMLG and PMDG, while that for PMDLG was at $620\text{--}630 \text{ cm}^{-1}$. Masuda, *et al.*,⁴ proposed to estimate the helix content from the peak intensity of the amide V band relative to that at 2950 cm^{-1} , and calculated the helix content of PMDLG from their experimental data.

The helix content estimated from the amide V intensity is the sum of the contents of the right- and left-handed α -helices, since the unpolarized infrared intensity is independent of



Figure 2. Infrared spectra for PMDLGs covering the whole range of copolymer compositions. Films were cast from chloroform—TFA(95:5 v/v) mixture, and their thickness were about 20μ . The code numbers in Figure 2 correspond to sample numbers in Table I.

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Table III. Infrared intensity ratio (615–630 cm⁻¹/2950 cm⁻¹) and X_{R+L}^H in solid films of PMDLG

Sample No.	Monomer ratio, L%	Intensity ratio	Sum of R- and L-Helix content, X_{R+L}^H
A-1	100.0	0.72	1.00
A-2	90.0	0.70	0.96
A-3	80.0	0.61	0.85
A-4	69.9	0.55	0.76
A-5	60.0	0.47	0.64
A-6	50.0	0.44	0.60
A-7	40.0	0.48	0.65
A-8	30.0	0.57	0.78
A-9	20.0	0.61	0.85
A-10	10.0	0.69	0.95
A-11	0.0	0.72	1.00
B-12	100.0	0.72	1.00
B-13	90.0	0.65	0.90
B-14	80.0	0.59	0.81
B-17	50.0	0.39	0.54

helical sense. The intensity ratios obtained were shown in the third column of Table III. This indicates that the helix content X_{R-L}^H of the copolymers PMDLG is lower than that of homopolymer PMLG or PMDG.

In the fourth column of Table III are shown the total helix contents as estimated from the intensity ratio by assuming that the helix content of PMLG (or PMDG) is equal to unity. Though these values may not be strictly equal to the absolute values of helix content (sum), they afford a measure of the relative helix content in solid films.

The infrared spectrum measured with a PMDLG (A-6) film cast from TFA solution showed exactly the same absorption frequencies as that from chloroform–TFA solution (number 6 in Figure 2). This fact shows that, although PMDLG as well as PMLG and PMDG exists in random coil conformation in TFA, it is converted into the helix conformation when the solvent is evaporated.

Optical Rotatory Dispersion

The results obtained in the optical rotatory dispersion measurements in *m*-cresol were analyzed by using Moffitt–Yang's plot¹⁰. Moffitt–Yang's parameters, a_0 and b_0 obtained are listed in Table IV. From the parameter b_0 , one ob-

Table IV. Moffitt–Yang parameters, a_0 and b_0 , and X_{R-L}^H of PMDLG in *m*-cresol at 25°C

Sample No.	Monomer ratio, L%	a_0	b_0	Difference in R- and L-Helix content, X_{R-L}^H
A-1	100.0	460	-575	1.00
A-2	90.0	400	-505	0.88
A-3	80.0	350	-435	0.76
A-4	69.9	260	-320	0.56
A-5	60.0	135	-145	0.25
A-6	50.0	15	-20	0.04
A-7	40.0	-155	155	-0.27
A-8	30.0	-270	325	-0.57
A-9	20.0	-345	415	-0.72
A-10	10.0	-425	500	-0.87
A-11	0.0	-470	565	-0.99
A-1 + A-11	80.0	280	-340	0.59
B-12	100.0	470	-570	0.99
B-13	90.0	455	-500	0.87
B-14	80.0	405	-430	0.75
B-15	70.0	360	-330	0.57
B-16	60.0	200	-140	0.24
B-17	50.0	25	-20	0.04

tains X_{R-L}^H , the difference between the right- and left-handed helix content from

$$X_{R-L}^H = \frac{b_0 - b_{0,c}}{b_{0,h} - b_{0,c}} \quad (2)$$

where, $b_{0,h}$ and $b_{0,c}$ denote the b_0 values for a perfect helix and a perfect coil, respectively. $b_{0,c}$ is generally close to zero, while $b_{0,h}$ gives an average value of -600 in several helicogenic solvents. Cassim and Taylor¹¹ have studied the effects of solvent on the optical rotatory dispersion parameters of PBLG and concluded that the value $b_{0,h}$ is a function of refractive index of the solvent and is not necessarily constant. It is assumed here that $b_{0,h} = -575$ in *m*-cresol, taking into account the minimum value in the measurements. Further, it was assumed that $b_{0,c} = 0$ from the authors' previous data¹² in DCA. From the values of b_0 , the values X_{R-L}^H are listed in Table IV are obtained. In Figure 3, the Moffitt–Yang parameter b_0 is plotted against the copolymer composition. The b_0 value for an 80:20 blend of PMLG and PMDG fell on the straight line connecting b_0 's for both homopolymers. Table IV also indicates

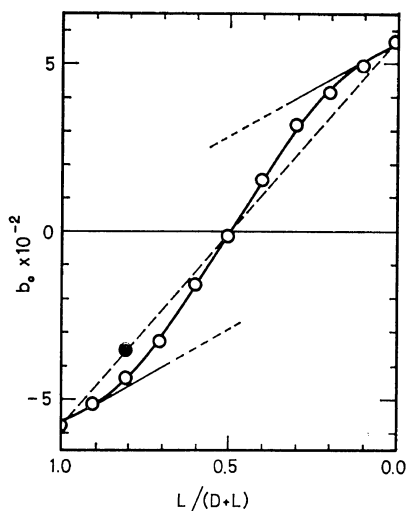


Figure 3. Copolymer composition dependence of Moffitt—Yang's parameter b_0 of PMDLGs in *m*-cresol at 25°C. The copolymer concentration is 1.0 g/dl. The filled circle denotes the value obtained with an 80:20 blend of PMLG and PMDG in *m*-cresol.

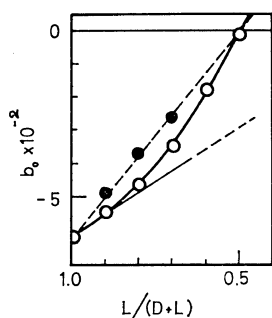


Figure 4. Copolymer composition dependence of Moffitt—Yang's parameter b_0 of PMDLGs in DCA(10 vol%)-DCE(90 vol%) mixtures at 25°C. The copolymer concentration is 1.0 g/dl. Filled circles denote values obtained with blends of PMLG(A-1) and PMDG(A-11).

that the difference in molecular weight may not significantly affect the difference in helix contents.

In another helicogenic solvent, DCA (10 vol%)—DCE (90 vol%) mixture, X_{R-L}^H was estimated from eq 2, in which it was assumed that $b_{0,h} = -615$ and $b_{0,c} = 0$. The data for some blends of PMLG and PMDG were also measured in

the same helicogenic solvent. b_0 is plotted against the copolymer composition in Figure 4. In this case, the b_0 values for blends of PMLG and PMDG fall on the straight line connecting b_0 's for both homopolymers. Masuda, *et al.*,⁴ have reported the optical rotatory dispersion of PMDLGs in chloroform. X_{R-L}^H values obtained by them⁴ are almost equal to the corresponding values in this paper. From these results it was concluded that X_{R-L}^H is not affected by the type of helicogenic solvent.

Estimation of Right-Handed Helix, Left-Handed Helix, and Random Coil Contents of PMDLG in Solution

As mentioned above, the infrared peak intensity of the amide V band relative to the 2950 cm^{-1} band gives the total helix content, *i.e.*, the sum of right-handed helix contents of PMDLG in solid films. Though the sum of right- and left-handed helix content in *m*-cresol cannot be estimated experimentally, it was tentatively assumed that the helix content estimated from IR data obtained with solid film is available even when the polymers are in helicogenic solvent. Using such an assumption the contents of right-handed helix, left-handed helix, and random coil were estimated. The results are plotted in Figure 5. For example, with respect to the equimolar-D, L-copolymer ($L=50\%$), the ratio of content, L-helix:R-helix:

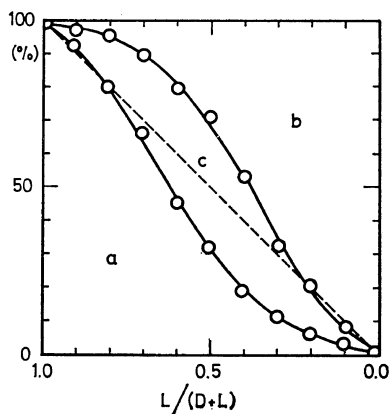


Figure 5. Contents of right-handed helix(a), left-handed helix(b), and random coil(c) of PMDLGs *m*-cresol at 25°C. Data are calculated from these of Tables III and IV.

random coil, is 29:31:40. The contents of both right- and left-handed helix reported by Masuda, *et al.*,⁴ are larger than the authors' values at corresponding copolymer compositions. Masuda, *et al.*, prepared PMDLG by polymerizing NCA's with sodium methoxide in dioxane. Sodium methoxide, as well as TEA, is known to be effective in preparing polypeptides of very high molecular weight. However, the former is called¹³ a "strong base" ("acylate ion") catalyst and the latter an "aprotic base" catalyst. Therefore, it seems that the polymerization mechanism with those two types of initiator may be different in nature¹³. The difference in helix content between both experiments, therefore, may be attributed to the effects of the kind of initiator and solvent.

Effect of Solvent Composition on Transition

In order to test the helix stability of the PMDLG in comparison with that of the homopolymer, the optical rotatory dispersion of these copolypeptides was examined in DCA—DCE mixtures as functions of solvent composition. Figure 6 shows the relation between the solvent composition and b_0 for these copolypeptides

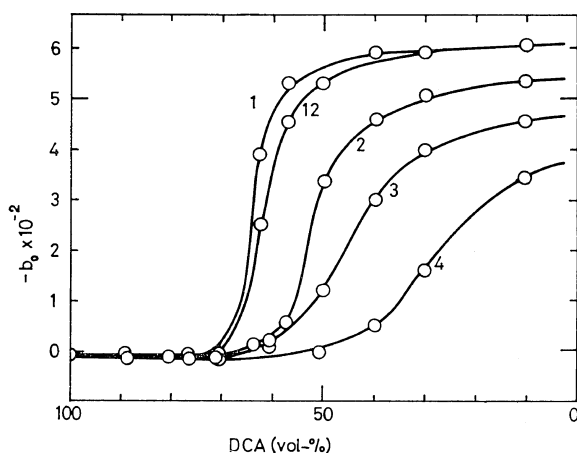


Figure 6. Effect of solvent composition on coil-to-helix transition at 15°C in DCA—DCE mixtures, for (1) PMLG [sample A-1, $[\eta]=2.78(\text{dl/g})$], (2) PMDLG($L=90 \text{ mol}\%$) [sample A-2, $[\eta]=1.38(\text{dl/g})$], (3) PMDLG($L=80 \text{ mol}\%$) [sample A-3, $[\eta]=1.08(\text{dl/g})$], (4) PMDLG($L=69.9 \text{ mol}\%$) [sample A-4, $[\eta]=0.97(\text{dl/g})$], and (12) PMLG [sample B-12, $[\eta]=0.97(\text{dl/g})$]. The polymer concentration is 1.0 g/dl.

and for homopolymer PMLG. It should be emphasized that the DCA contents where the b_0 value departs from $b_0=0$ are almost the same for all of these copolypeptides and PMLG. At lower DCA contents, however the highest values of b_0 are different from each other. This behavior is reasonable because X_{R-L}^H , determined by optical rotatory dispersion measurement, is the difference between the right- and left-handed helix content. Figure 6 also illustrates the fact that different amounts of DCA are required to destroy helical conformation at a given temperature. As the molar ratio of the D-isomer was increased, less DCA needed to be added to the DCE solution to bring about the helix destruction and, in addition, the transition was broadened. Both these observations indicate a decrease in helical stability. In the molecular weight range ($[\eta]=0.97-2.79$) of the samples used here, differences in molecular weight probably do not significantly affect the transition behavior.

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