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Chain Conformations of Polypeptide Copolymers

By

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Chain conformations have been investigated theoretically by assuming Markoffian process for copolypeptides composed of two components of L-alanine, glycine, *N*-methyl glycine and *N*-methyl-L-alanine, for D,L-copolyalanine, and for D,L-copoly-*N*-methyl alanine. The characteristic ratio of these copolymers was plotted against the sequence probability that a residue is followed by a residue of the same kind. Some experimental results obtained for equimolar-D,L-copoly-γ-methyl glutamate are discussed with the aid of theoretical equation.

I. Characteristic Ratios of Two-Component Copolypeptides

In section I, we deal with the characteristic ratios for copolypeptides composed of two components of L-alanine (Ala), glycine (Gly), *N*-methyl glycine (NMGly), and *N*-methyl-L-alanine (NMAla), for D, L-copolyalanine, and for D, L-copoly-*N*-methyl alanine.

The characteristic ratios of copolypeptides first were calculated by Flory and his coworkers for random Gly-Ala type copolymers,[1] D,L-Ala type copolymers[1], and copolymers containing proline residues.[2] *N*-substituted amino acid residues are different from α-amino acid residues in that the former form imide bonds in the chain and thus can not form intramolecular hydrogen bonds in contrast to amide bonds formed by the latter.

The nmr studies on imide bonds of poly-*N*-methyl-L-alanine (high polymer) by Goodman[3] have disclosed the presence of only *trans* imide bonds, but those for poly-*N*-methyl glicine (high polymer) by Bovey[4] showed that the polymer contains both *cis*- and *trans*-imide bonds. We shall first be concerned with the random chain conformation by assuming *trans* conformation for all the amide and imide bonds included

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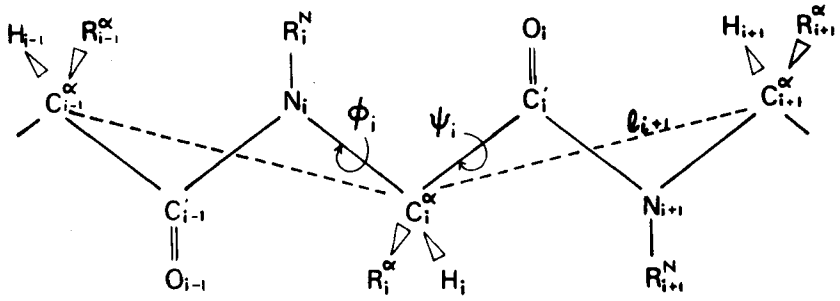
in copolymer chains. The effect of the presence of *cis* imide bonds in poly-*N*-methyl glycine will be discussed separately.

1. Conformational Energy Contour Diagrams



A portion of a chain including the i-th amino acid residue and parts of both the $(i-1)$-th and $(i+1)$-th amino aicd residue is represented in the figure, in which R_i^N and R_i^α denote, respectively, the substituents attached to the nitrogen atom N_i and to α-carbon atom C_i^α of the i-th residue. By assigning either CH_3 or H for R_i^N, R_i^α and R_{i+1}^N, we have homopolymers and copolymers including Gly, Ala, NMGly and/or NMAla. Positive values of φ and ψ are assigned for right-handed rotations, and negative values for left-handed rotations. Thus, D,L-copolymers are also taken into account. For NMGly or NMAla, one of the hydrogen atoms in $R_i^N(=CH_3)$ is fixed at *trans* position against C_{i-1}'.

The conformational energy calculations were carried out using well-established methods in accordance with Flory[5] and Scheraga[6]. To estimate the conformational energy of the i-th residue in a copolymer chain, the substituent R_{i+1}^N of the $(i+1)$-th residue should be taken into account. Thus, the conformational energy represented by $V(R_i^N, R_i^\alpha, R_{i+1}^N)$ (φ_i, ψ_i) was calculated for eight combinations shown in Table I, from the sum of three contributions: intrinsic torsional potential energy $V_{(T)}$ (φ) and $V_{(W)}$ (ψ), the sum $\sum V_{(T)jk}$ of van der Waals interaction energies between all atoms or groups j and k, and dipolar interaction energies $\sum V_{(D)ik}$ between amide and amide, amide and imide, imide and imide, or imide and amide groups.

$$V(R_i^N, R_i^\alpha, R_{i+1}^N)(\varphi, \psi) = V_{(T)}(\varphi) + V_{(T)}(\psi) + \sum V_{(W)jk}(\varphi, \psi) + \sum V_{(D)ik}(\varphi, \psi) \quad (1)$$

Values of molecular parameters such as bond length and bond angle, and torsional potential functions used here are the same as those used by Flory[5]. The van der Waals interactions between all pairs of non-bonded atoms in the dipeptide sequence were

Table 1.

	$-\overset{R_i^N}{\underset{}{N}}-\overset{R_i^\alpha}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{R_{i+1}^N}{\underset{}{N}}-$	$R_i^N \cdot R_i^\alpha \cdot R_{i+1}^N$
Gly-Gly Gly-Ala	$-\overset{H}{\underset{}{N}}-\overset{H}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{}{N}}-$	H·H·H
Ala-Ala Ala-Gly	$-\overset{H}{\underset{}{N}}-\overset{CH_3}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{}{N}}-$	H·CH_3·H
NMGly-NMGly NMGly-NMAla	$-\overset{CH_3}{\underset{}{N}}-\overset{H}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\underset{}{N}}-$	CH_3·H·CH_3
NMAla-NMAla NMAla-NMGly	$-\overset{CH_3}{\underset{}{N}}-\overset{CH_3}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\underset{}{N}}-$	CH_3·CH_3·CH_3
NMAla-Ala NMAla-Gly	$-\overset{CH_3}{\underset{}{N}}-\overset{CH_3}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{}{N}}-$	CH_3CH_3H
Ala-NMGly Ala-NMAla	$-\overset{H}{\underset{}{N}}-\overset{CH_3}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\underset{}{N}}-$	H·CH_3·CH_3
NMGly-Ala NMGly-Gly	$-\overset{CH_3}{\underset{}{N}}-\overset{H}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{}{N}}-$	CH_3·H·H
Gly-NMGly Gly-NMAla	$-\overset{H}{\underset{}{N}}-\overset{H}{\underset{}{CH}}-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\underset{}{N}}-$	H·H·CH_3

estimated using[6] Lennard-Jones "6-12" potential functions. The van der Waals radii of the atoms and groups were cited from Flory's paper[5] except the value for β-methylene group, which was given as $r_{CH_2}=2.00$Å, the value recommended for methyl group by Pauling[7]. The dipolar interaction energy was calculated by point monopole charge approximation with the use of values of partial charges obtained by Ooi and Scheraga[9] for imide bond (Table 2).

The conformational energy was calculated at 20° intervals from 0° to 360° for φ and ψ. Figs. 1–8 show the contour diagrams for eight kinds of sequence given in Table 1.

The contour map for Gly-Gly (or Gly-Ala) (Fig. 1) is centrosymmetric and almost the same as that reported by Brant and Flory[10]. The energy minima locate at $\varphi=100°$, $\psi=260°$, and at $\varphi=260°$, $\psi=100°$. The energy minimum for Ala-Ala (or Ala-

Table 2. Partial Charges on amide and imide groups

Atom	Amide[8]	Imide[9]
H′	0.272	
N	−0.305	−0.282
C′	0.449	1.056
O	−0.416	−1.240
C		0.165
H_1 (*trans* for C′)		0.020
H_2		0.022
H_3		0.022

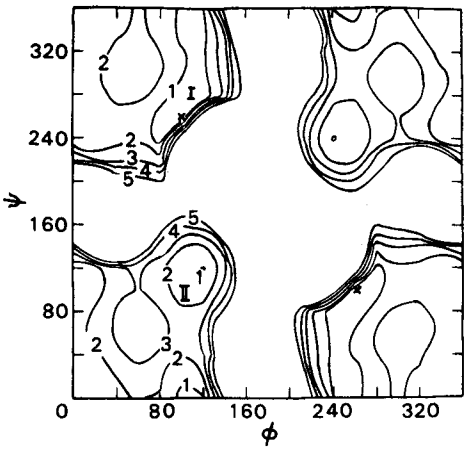


Fig. 1. Contour diagrams of total conformational energy for Gly-Gly or Gly-Ala. The absolute minima indicated by × correspond to −3.40 kcal/mol. The numerals on the contours are in the units kcal/mol relative to this minimum.

Gly) (Fig. 2) locates at $\varphi=20°$, $\psi=340°$ in region I, in comparison with $\varphi=100°$, $\psi=330°$ given by Brant *et al.*[10] This difference is attributed to the difference in the van der Waals radius of β-methylene group ($r_{CH_2}=1.85$ Å, Brant). The contour map of NMGly-NMGly (or NMGly-NMAla) (Fig. 3) is centrosymmetric and energy minima locate at $\varphi=100°$, $\psi=0°$, and at $\varphi=260°$, $\psi=0°$. The contour map of NMAla-NMAla (or NMAla-NMGly) (Fig. 4) indicates that rotations about φ and ψ are extremely restricted. The absolute minimun locates at $\varphi=240°$, $\psi=260°$, which is very close to $\varphi=238°$, $\psi=227°$ assigned for left-handed α-helix. Goodman[11,12] and Liquori[13] have reported the conformational energy for this sequence with the use of potential functions different from ours and without taking into account the dipolar interactions.

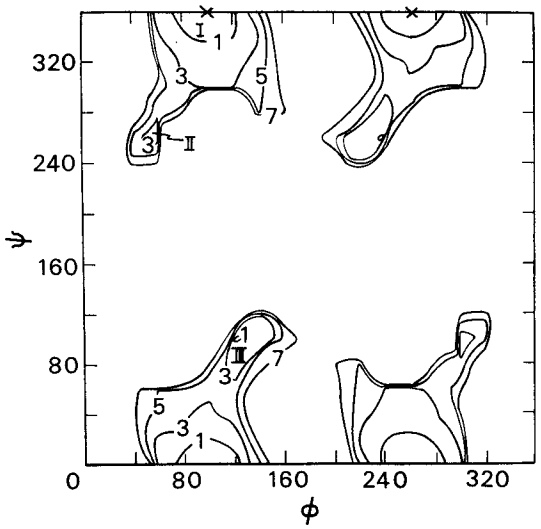


Fig. 2. Contour diagrams of total conformational energy for Ala-Ala or Ala-Gly. The absolute minimum indicated by × corresponds to −1.47 kcal/mol.

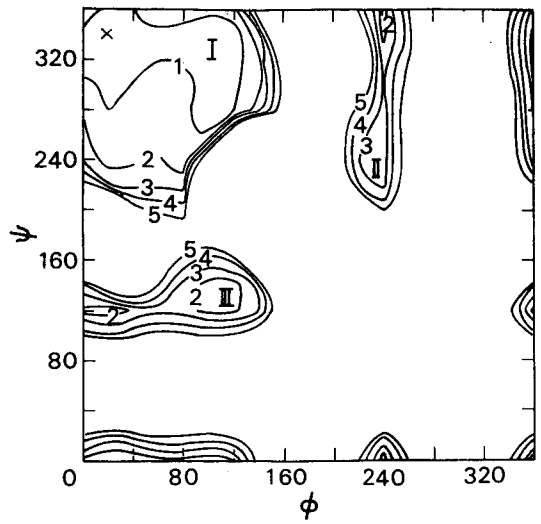


Fig. 3. Contour diagram of total conformational energy for NMGly-NMGly or NMGly-NMAla. The absolute minima indicated by × correspond to −3.46 kcal/mol.

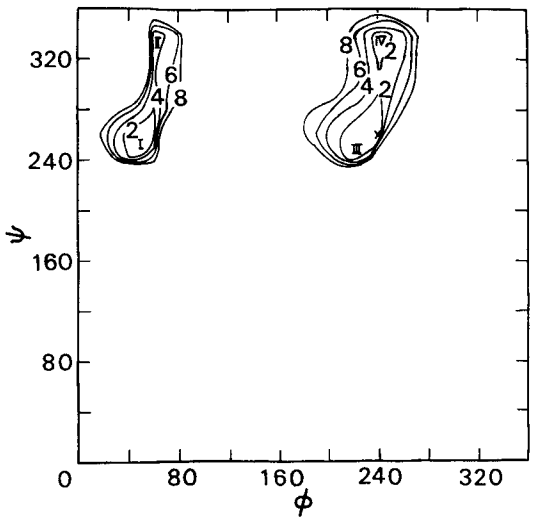


Fig. 4. Contour diagram of total conformational energy for NMAla-NMAla or NMAla-NMGly. The absolute minimum indicated by × corresponds to 0.18 kcal/mol.

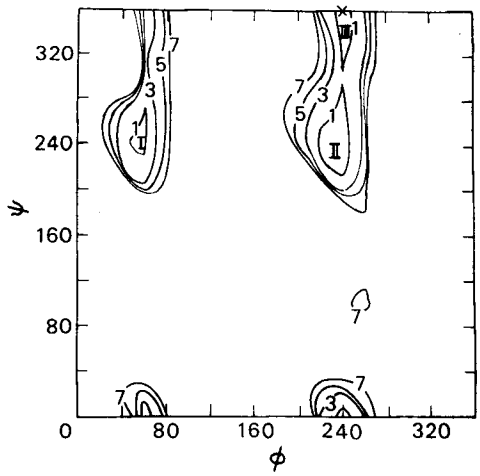


Fig. 5. Contour diagram of total conformational energy for NMAla-Ala or NMAla-Gly. The absolute minimum at × corresponds to −0.39 kcal/mol.

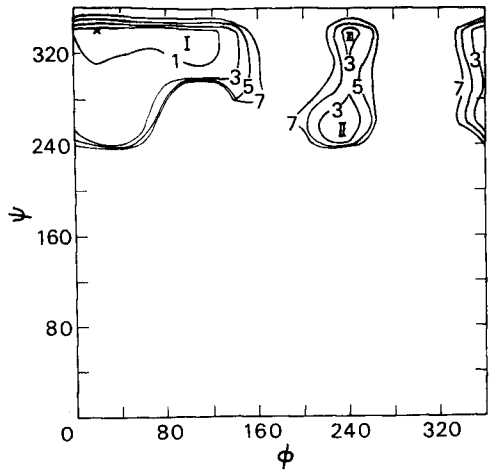


Fig. 6. Contour diagram of total conformational energy for Ala-NMGly or Ala-NMAla. The absolute minimum at × corresponds to 1.25 kcal/mol.

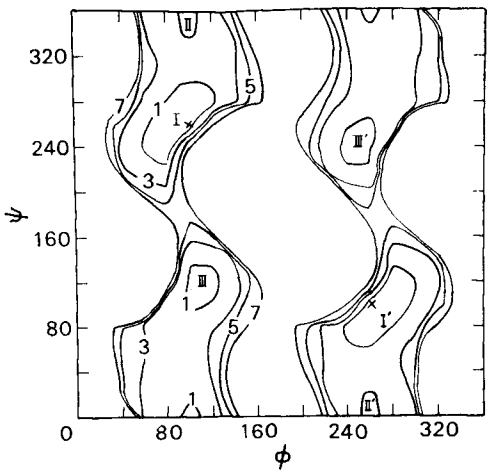


Fig. 7. Contour diagram of total conformational energy for NMGly-Ala or NMGly-Gly. The absolute minima at × correspond to −4.14 kcal/mol.

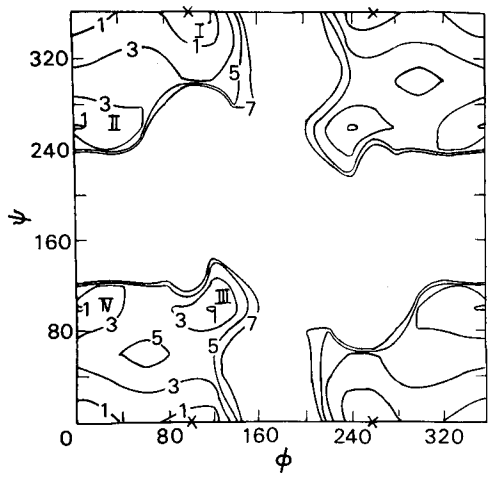


Fig. 8. Contour diagram of total conformational energy for Gly-NMGly or Gly-NMAla. The absolute minima at × correspond to −1.34 kcal/mol.

Goodman has pointed out that the right-handed three-fold helix or slightly distorted left-handed helix seems to represent the most stable conformation.

In the following, the contour maps shown in Figs. 1–8 are used to obtain characteristic ratios of homopolypeptides and copolypeptides.

2. Characteristic Ratios of Homopolypeptides

With the use of the contour maps shown in Figs. 1–4, the averaged transformation matrices $\langle \boldsymbol{T} \rangle$ for Gly-Gly, Ala-Ala, NMGly-NMGly, and NMAla-NMAla were calculated as

$$\langle \boldsymbol{T} \rangle_{\mathrm{H\cdot H\cdot H}} = \begin{bmatrix} 0.368 & -0.004 & 0 \\ -0.163 & -0.392 & 0 \\ 0 & 0 & -0.140 \end{bmatrix} \tag{2}$$

$$\langle \boldsymbol{T} \rangle_{\mathrm{H\cdot CH_3\cdot H}} = \begin{bmatrix} 0.565 & 0.370 & 0.499 \\ 0.055 & -0.573 & 0.417 \\ 0.566 & -0.417 & 0.330 \end{bmatrix} \tag{3}$$

$$\langle \boldsymbol{T} \rangle_{\mathrm{CH_3\cdot H\cdot CH_3}} = \begin{bmatrix} 0.578 & 0.653 & 0 \\ -0.267 & -0.032 & 0 \\ 0 & 0 & 0.146 \end{bmatrix} \tag{4}$$

$$\langle \boldsymbol{T} \rangle_{\mathrm{CH_3\cdot CH_3\cdot CH_3}} = \begin{bmatrix} 0.332 & 0.132 & 0.835 \\ -0.166 & 0.552 & -0.222 \\ -0.557 & -0.027 & 0.223 \end{bmatrix} \tag{5}$$

The characteristic ratios of homopolymers were calculated for chains with infinite chain

Table 3. Characteristic Ratios of Homopolypeptides

Polymer	$\langle R_0^2 \rangle / nl^2$	
	Calc	Obs
Polyglycine	2.17	—
Poly-L-alanine	8.38	(8.6) av
Poly-*N*-methyl glycine	2.97	1.8±0.2*
Poly-*N*-methyl-L-alanine	0.58	—

* ref. 14)

length by the well-established method[5] (Table 3). For polyglycine, experimental data are not available. The observed value for polyalanine was denoted by an average value obtained by various glutamate esters. The observed value for poly-*N*-methyl glycine was obtained from Fessler's data[14] by Stockmayer-Fixman's plot. The calculated value 2.97 was obtained by assuming *trans* conformation for all imide bonds. In a separate paper[15], we have examined the effect of *cis* (*trans*) residues discretely located in *trans* (*cis*) sequence for polyisoprene. Calculations for poly-*N*-methyl glycine are under investigation. The observed value (1.8±0.2) may suggest the presence of both *trans* and *cis* residues as pointed out by Bovey[4]. The calculated characteristic ratio 0.58 for poly-*N*-methyl-L-alanine is unusually small though the potential minimum for NMAla-NMAla locates at angles near those assigned for left-handed α-helix conformation. The chain may be regarded as a compact coil.

3. Characteristic Ratios of Copolypeptides

Let p_{AB} be the conditional probability that an A residue is succeeded by a B residue, and assume that p_{AB}, p_{AA}, p_{BA}, and p_{BB} are constant in the course of polymerization process, then the matrix $\boldsymbol{P}$ of sequence probability for the i-th amino acid residue is given by

$$\boldsymbol{P}_i = \begin{bmatrix} p_{AA} & p_{AB} \\ p_{BA} & p_{BB} \end{bmatrix} \tag{6}$$

and for $i=1$

$$\boldsymbol{P}_1 = \begin{bmatrix} p_A & 0 \\ 0 & p_B \end{bmatrix} \tag{7}$$

where p_A and p_B are *a priori* probabilities for A and B, respectively. Further $p_A p_{AA} + p_B p_{BA} = p_A$.

The binary copolypeptides investigated here are classified into two categories, type I and type II: copolymers for which the conformational energy $V(\varphi_i, \psi_i)$ for the i-th

residue is not varied by the selection of monomers (A or B) for $(i+1)$-th residue are denoted as type I, while those for which $V(\varphi_i, \psi_i)$ is varied are denoted as type II.

The characteristic ratios of type I copolypeptides with finite chain length were calculated by the method established by Miller *et al*[1]. With type II copolypeptides, the following considerations were taken in account.

Since the conformational energy of the i-th residue depends on the monomer selection for the $(i+1)$-th residue, $\boldsymbol{G}_i$ should be constructed individually for AA, AB, BA, and BB (*i. e.*, $\boldsymbol{G}_{AA}$, $\boldsymbol{G}_{AB}$, $\boldsymbol{G}_{BA}$, $\boldsymbol{G}_{BB}$).

$$\boldsymbol{G}_i = \begin{bmatrix} 1 & \boldsymbol{l}^T\langle \boldsymbol{T} \rangle & \boldsymbol{l}^2/2 \\ \boldsymbol{0} & \langle \boldsymbol{T} \rangle & \boldsymbol{l} \\ \boldsymbol{0} & \boldsymbol{0} & 1 \end{bmatrix} \tag{8}$$

Now we construct a matrix $\boldsymbol{K}$ with the use of matrices $\boldsymbol{G}$'s and $\boldsymbol{P}_i$. It should be noted that, for type II copolypeptides, the reactivity of the i-th residue depends on the kind of the $(i-1)$-th residue, but the conformation of the i-th residue depends on the kind of the $(i+1)$-th residue. Accordingly, the matrix $\boldsymbol{K}$ for the i-th residue should be constructed after the reaction proceeds to the $(i+1)$-th residue. In other words, $\boldsymbol{P}$ introduced in $\boldsymbol{K}$ should be $\boldsymbol{P}_{i+1}$ instead of $\boldsymbol{P}_i$. Thus, for the i-th residue $(1<i<n)$,

$$\boldsymbol{K} = \begin{bmatrix} \boldsymbol{G}_{AA}\, p_{AA} & \boldsymbol{G}_{AB}\, p_{AB} \\ \boldsymbol{G}_{BA}\, p_{BA} & \boldsymbol{G}_{BB}\, p_{BB} \end{bmatrix} \tag{9}$$

and for $i=1$,

$$\boldsymbol{K}_1 = \begin{bmatrix} \boldsymbol{E}_5\, p_A & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{E}_5\, p_B \end{bmatrix} \tag{10}$$

where $\boldsymbol{E}_5$ is the unit matrix of the order 5. For the terminal residue $(i=n)$, the matrix $\boldsymbol{K}_n^*$ is written as

$$\boldsymbol{K}_n^* = \boldsymbol{K}_{n-1} \begin{bmatrix} \boldsymbol{G} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{G} \end{bmatrix} = \begin{bmatrix} \boldsymbol{G}_{AA}\, p_{AA}\, \boldsymbol{G} & \boldsymbol{G}_{AB}\, p_{AB}\, \boldsymbol{G} \\ \boldsymbol{G}_{BA}\, p_{BA}\, \boldsymbol{G} & \boldsymbol{G}_{BB}\, p_{BB}\, \boldsymbol{G} \end{bmatrix} \tag{11}$$

Finally, the characteristic ratio is expressed by

$$\frac{\langle \overline{R_0^2} \rangle}{nl^2} = \left(\frac{2}{nl^2}\right) [1\ 0\ 0\ 0\ 0\ 1\ 0\ 0\ 0\ 0]\, \boldsymbol{K}_1\, \boldsymbol{K}^{n-2}\, \boldsymbol{K}_n^* \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix} \tag{12}$$

The calculated average transformation matrices $\langle T \rangle$'s for four sequences other than those given in Eqs. (2)-(5) were as follows:

$$\langle T \rangle_{CH_3 \cdot CH_3 \cdot H} = \begin{bmatrix} 0.340 & 0.156 & 0.492 \\ -0.250 & 0.366 & -0.277 \\ -0.509 & 0.118 & 0.205 \end{bmatrix} \tag{13}$$

$$\langle T \rangle_{H \cdot CH_3 \cdot CH_3} = \begin{bmatrix} 0.579 & 0.328 & 0.646 \\ 0.043 & -0.658 & 0.232 \\ 0.601 & -0.127 & -0.359 \end{bmatrix} \tag{14}$$

$$\langle T \rangle_{CH_3 \cdot H \cdot H} = \begin{bmatrix} 0.251 & -0.183 & 0 \\ -0.313 & -0.305 & 0 \\ 0 & 0 & -0.115 \end{bmatrix} \tag{15}$$

$$\langle T \rangle_{H \cdot H \cdot CH_3} = \begin{bmatrix} 0.620 & 0.615 & 0 \\ -0.097 & -0.212 & 0 \\ 0 & 0 & -0.047 \end{bmatrix} \tag{16}$$

The characteristic ratios for type I and type II copolypeptides were calculated with the use of appropriate equations from Eqs. (2)–(5) and Eqs. (13)–(16).

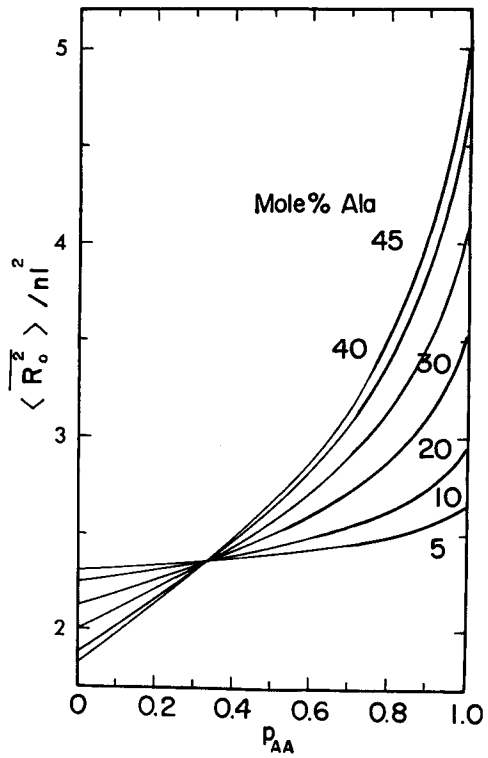


Fig. 9. Plot of the characteristic ratio against the sequence probability p_{AA} for Ala(A)-NMGly(B) copolymers (n=153). The numerals on the curves denote the mole% of A (minor component).

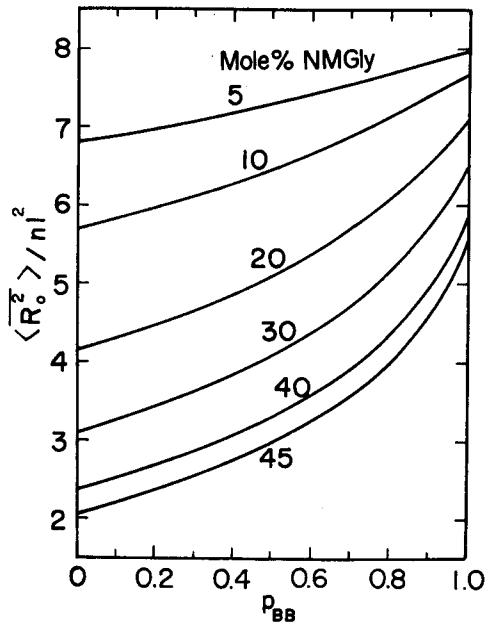


Fig. 10. Plot of the characteristic ratio against the sequence probability p_{BB} for Ala(A)-NMGly(B) copolymers ($n=153$). The numerals on the curves denote the mole% of B (minor component).

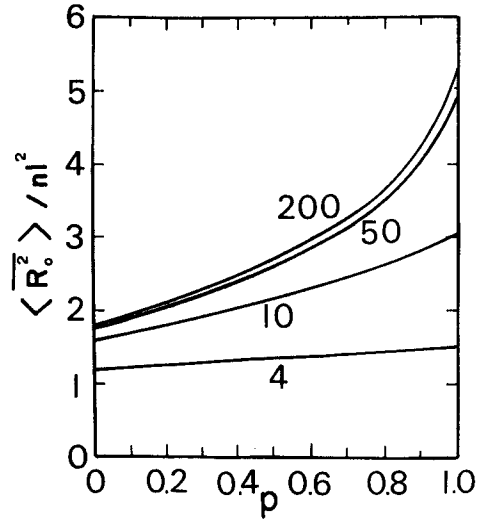


Fig. 11. Plot of the characteristic ratio against the sequence probability p ($=p_{AA}=p_{BB}$) for equimolar Ala(A)-NMGly(B) copolymers. The numerals on the curves denote the number of virtual bonds, n, in the chain.

Details of the results of calculations will be reported shortly in Polymer Journal.[16] Here some of them will be given for Ala-NMGly copolymers (Figs. 9-11), Ala-NMAla copolymer (Figs. 12–13), Ala-Gly copolymers (Fig. 14), and NMAla-NMGly copolymers and others (Fig. 15).

With respect to Ala-NMGly copolymers, it is pointed out that the characteristic ratio (1.8) of the alternating copolymer ($p=0$) with $n=200$ (Fig. 11) is less than those, 8.4 and 2.97, for Ala-homopolymer and NMGly-homopolymer. Conformation of the alternating copolymer is given merely by Ala-NMGly and NMGly-Ala sequence, and no longer affected by NMGly-NMGly and Ala-Ala sequence which contribute to both homopolymers. For this reason, the characteristic ratio of the alternating Ala-NMGly copolymer differs from those of component homopolymers. The contour maps for Ala-NMGly (Fig. 6) and for NMGly-Ala (Fig. 7) show that the conformationally preferred regions locate at skewed conformations, resulting in such a small value as 1.8 for

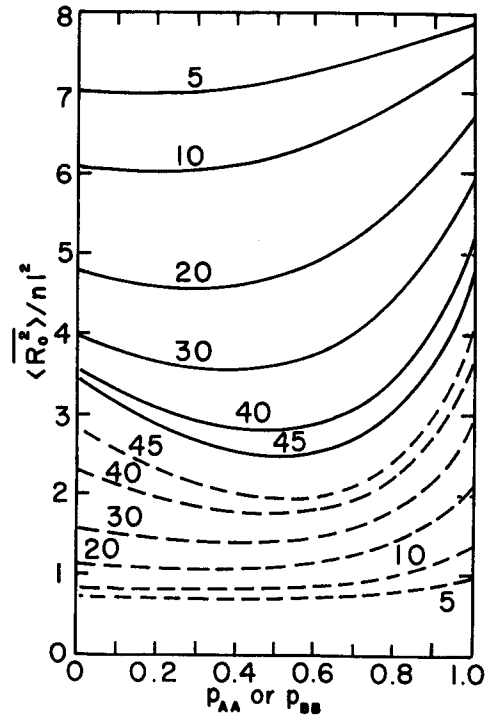


Fig. 12. Plot of the characteristic ratio against the sequence probability p_{AA} or p_{BB} for Ala(A)-NMAla(B) copolymers ($n=153$). Solid curves: NMAla(B) is the minor component; numerals on the curves are mole% of NMAla(B); abscissa is p_{BB}. Broken curves: Ala(A) is the minor component; numerals on the curves are mole% of Ala(A); abscissa is p_{AA}.

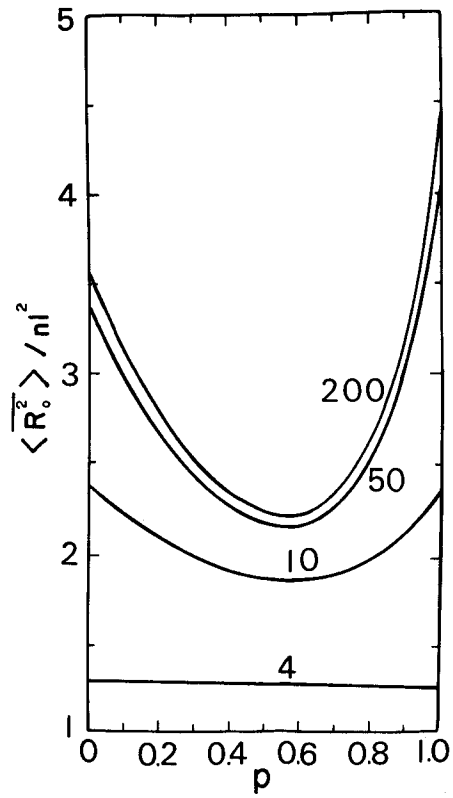


Fig. 13. Plot of the characteristic ratio against the sequence probability p ($=p_{AA}=p_{BB}$) for equimolar Ala(A)-NMAla(B) copolymers. The numerals on the curves denote the number of virtual bonds, n, in the chain.

characteristic ratio of the alternating copolymer. The characteristic ratios in the range of $0.5<p<1$ monotonously increases with increasing p, *i.e.*, with increasing blockiness. Such behavior may originate from extended conformation for Ala-Ala (Fig. 2) sequence.

On the other hand, the characteristic ratio of alternating Ala-NMAla copolymer with $n=200$ (Fig. 13) is as large as 3.5. This case is explained with contour maps for Ala-NMAla (Fig. 6) and NMAla-Ala (Fig. 5). In Fig. 6, the energy minimum locates at $\varphi=20°$, $\psi=340°$, which corresponds to a rather extended conformation. Further, in Fig. 5 the minimum locates at $\varphi=240°$, $\psi=0°$, which corresponds to G'T conformation. These two contributions may reflect the rather large characteristic ratio for the alternating copolymer. The characteristic ratios in the range of $0<p<0.5$ decrease with increasing p. This may be due mainly to the fact that the influence of NMAla-NMAla sequence (Fig. 4) is large. In the range of $0.5<p<1$, the charac-

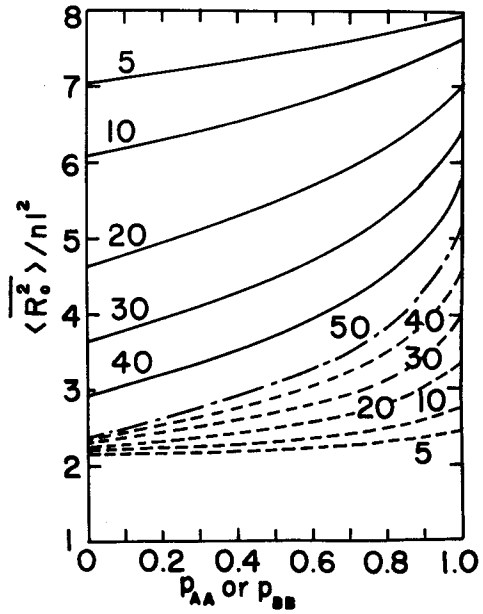


Fig. 14. Plot of the characteristic ratio against the sequence probability p_{AA} or p_{BB} for Ala(A)-Gly(B) copolymers ($n = 153$). Solid curves: Gly(B) is the minor component; numerals on the curves are mole% of Gly(B); abscissa is p_{BB}. Broken curves: Ala(A) is the minor component; numerals on the curves are mole% of Ala(A); abscissa is p_{AA}.

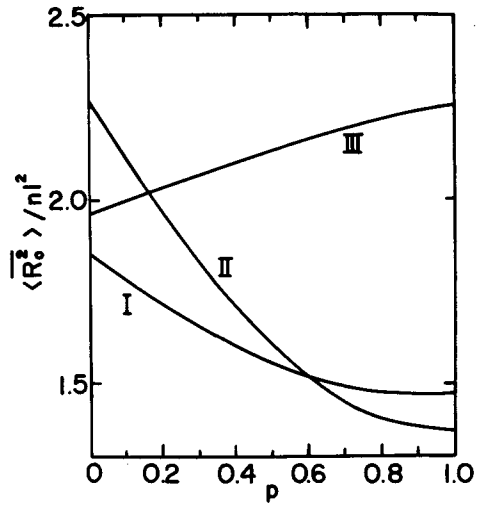


Fig. 15. Plot of the characteristic ratio against the sequence probability p ($=p_{AA}=p_{BB}$) for equimolar NMAla-NMGly copolymers (curve I), equimolar Gly-NMAla copolymers (curve II), and equimolar Gly-NMGly copolymers (curve III).

teristic ratios increase with increasing p, which is attributed to that extended conformations are preferred for Ala-Ala sequence (Fig. 2).

4. Characteristic Ratios of D,L-Copolypeptides

D,L-Copolypeptides belong to type I. The conformational energy of D-isomer is related to that of L-isomer by $V_L(\varphi, \psi) = V_D(-\varphi, -\psi)$. Thus the average transformation matrices for D·Ala-D·Ala and for D·NMAla-D·NMAla were derived from Eqs. (3) and (5), respectively, as

$$\langle T \rangle_{D-(H\cdot CH_3\cdot H)} = \begin{bmatrix} 0.565 & 0.370 & -0.499 \\ -0.055 & -0.573 & -0.417 \\ -0.566 & 0.417 & -0.330 \end{bmatrix} \tag{17}$$

$$\langle T \rangle_{D-(CH_3\cdot CH_3\cdot CH_3)} = \begin{bmatrix} 0.332 & 0.132 & -0.835 \\ -0.166 & 0.552 & 0.221 \\ 0.557 & 0.027 & 0.223 \end{bmatrix} \tag{18}$$

In Figs. 16 and 17, the characteristic ratios are plotted against the sequence probability p(*i.e.*, p_{LL} or p_{DD}) that a residue of minor component is succeeded by the same kind of residue for D,L-polyalanine and D,L-poly-*N*-methyl alanine. Note

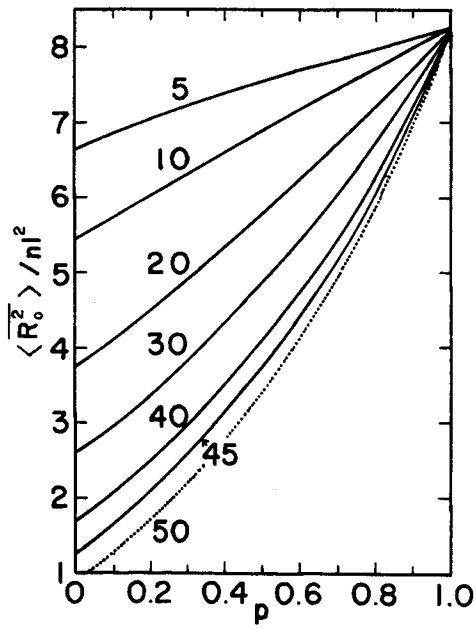


Fig. 16. Plot of the characteristic ratio against the sequence probability p that a residue of minor component is succeeded by the same kind residue for D,L-Ala copolymers ($n = 150$). The numerals on the curves denote the mole% of the minor component.

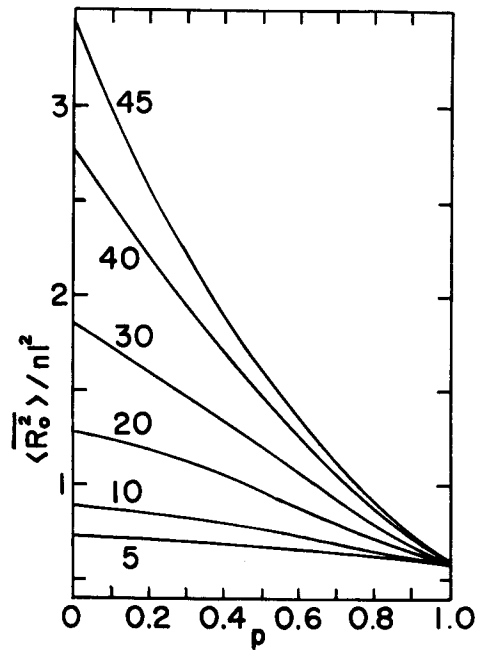


Fig. 17. Plot of the characteristic ratio against the sequence probability p that a residue of minor component is succeeded by the same kind residue for D,L-NMAla copolymers (n=150). The numerals on the curves denote the mole% of the minor component.

that p_{LL} is equal to p_{DD} for equimolar (racemic) D,L-copolymers but p_{LL} is not equal to p_{DD} for non-equimolar D,L-copolymers. These figures lead to the fact that the plot of characteristic ratio against the stereo composition (say % L-isomer) of random sequenced D,L-copolymers gives a symmetrical curve about an axis at % L=50. The result for equimolar D,L-Ala-copolymer (dotted curve in Fig. 16) is almost the same as that reported by Miller *et al.*[1] The characteristic ratio monotonously increases with p(Fig. 16). On the other hand, for D,L-NMAla-copolymer the characteristic ratio decreases with p. In alternating D,L-NMAla-copolymer, *i.e.*, in syndiotactic polymer, conformations correspond to mirror images of each other appearing alternating in the chain, which leads to a relatively large value (4.3) for characteristic ratio of this polymer at p=0 (Fig. 17). As the isotacticity p approaches 1, the average chain becomes compact coil peculiar to L- and D-homopolymers.

5. Effect of *cis*-Residues in *trans*-Sequence

In all the treatments mentioned above, amide and imide bonds were assumed to be fixed at *trans*. To take into account the effect of *cis*-bonds in *trans* sequence,

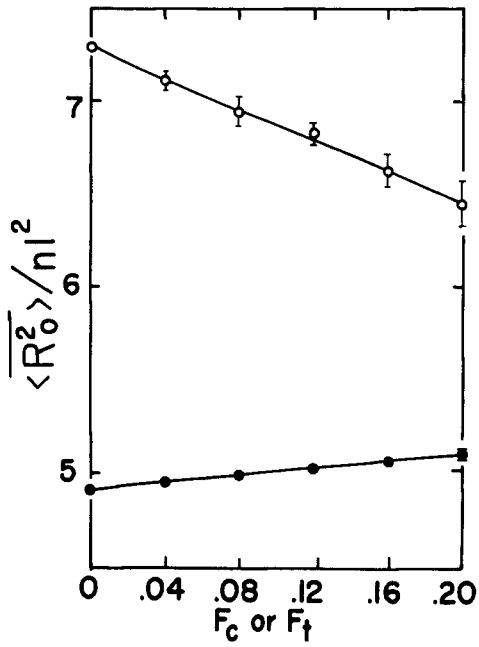


Fig. 18. Effect of the presence of discrete *cis* residues in *trans* chain (○), and *trans* residues in *cis* chain (●) for polyisoprene.

some calculations were carried out[15] for polybutadiene and polyisoprene. An example of the results is given in Fig. 18. Effect of *cis* in *trans* chain is rather remarkable. Recently, Abe and Flory[17] have independently reported some results on the same problem.

II. Experimental Studies on Chain Conformations of D,L-Copolypeptides

Among various copolymers mentioned above, equimolar-D,L-copoly-γ-methyl glutamate was investigated to obtain the characteristic ratio and to get information about the arrangement of D- and L-residues in the chains in connection with polymerization conditions.

The copolymers were prepared by polymerizing equimolar mixtures of D- and L-γ-methyl glutamate *N*-carboxyanhydrides in dioxane-methylene chloride 1 : 1 mixture at 25°C with triethyl amine (TEA) as the initiator. The monomer to initiator ratios, $[M]/[I]$, were varied from 50 to 500 to obtain samples with different molecular weights.

Intrinsic viscosities were measured at 25°C in dichloroacetic acid (DCA) and in

Table 4. Solution Properties of Equimolar-D,L-Copoly-γ-methyl glutamate

Sample	$[M]/[I]$	$[\eta]$ DCA	$[\eta]$ *m*-cresol	$M_n \times 10^{-4}$	$A_2 \times 10^4$ (ml.mol/g^2)
I	50	0.415	0.348	3.27	1.23
II	100	0.463	0.424	3.94	1.28
III	200	0.549	0.456	4.72	1.65
IV	250	0.614	0.509	5.00	1.32
V	300	0.634	0.555	5.13	1.31
VI	500	0.912	0.730	8.21	1.13

m-cresol by the use of cellophane membrane. The results obtained were shown in Table 4 and Fig. 19. DCA is known as a coil solvent for poly-γ-methyl-L-glutamate, and this is also true for the D,L-copolymer. Thus, we can estimate the random chain conformation from the relation between $[\eta]$ and the molecular weight. On the other hand, *m*-cresol is helicogenic solvent for D- or L-homopolymer. The obtained parameters in the viscosity-molecular weight equation, *i.e.*, the exponent value 0.78 less than 1, may suggest that the chains are not dispersed in rod-like form owing to the mode of arrangement of D and L residues in the chains. Further, the intrinsic viscosities of samples in *m*-cresol are lower than those in DCA.

Nagai[18] has derived an expression between the mean square radius of gyration

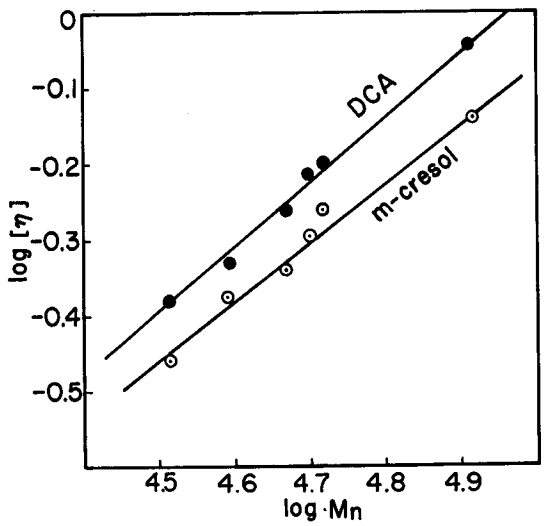


Fig. 19. Intrinsic viscosity $[\eta]$ in dichloroacetic acid (DCA) and in *m*-cresol plotted against number-average molecular weight M_n, on double logarithmic scale, for equimolar-D,L-poly-γ-methyl glutamate.
$[\eta]=5.9\times10^{-5}M_n^{0.85}$ in DCA, $[\eta]=1.1\times10^{-4}M_n^{0.78}$ in *m*-cresol.

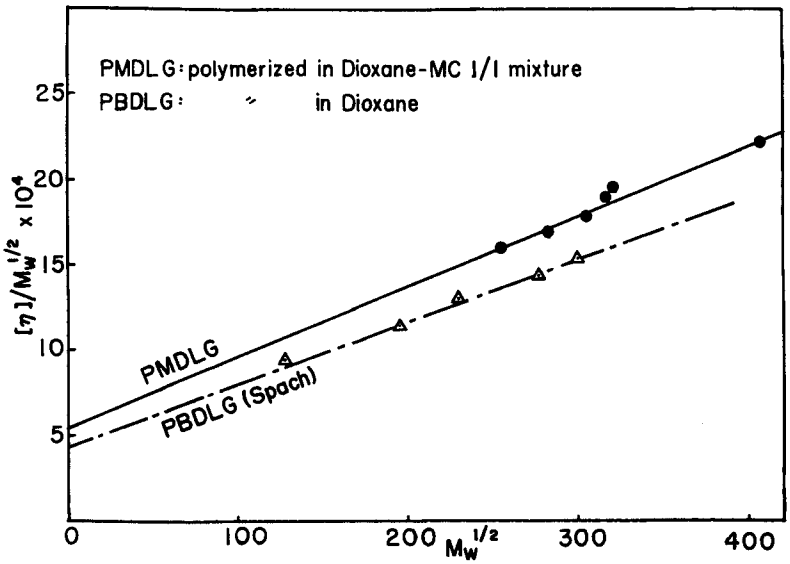


Fig. 20. $[\eta]/M_w^{1/2}$ plotted against $M_w^{1/2}$ for equimolar-D,L-poly-γ-methyl glutamate (PMDLG) and for equimolar-D,L-poly-γ-benzyl glutamate (PBDLG) (Spach's data). $[\eta]$'s are the values in DCA.

$\langle S^2 \rangle$ and the helical content f for polypeptides consisting of alternating sequences of helical and randomly coiled sections. Nagai's expression leads to the fact that with increasing f, the chain expansion first decreases, passes through a minimum at about $f=0.5$, and then markedly increases.

Actually, the viscosity data on D,L-copolymers in *m*-cresol may suggest that the D and L residues are so arranged in the chains as to form alternating sequences of helical and coiled sections. This point will be further discussed later.

Now we return to the coil conformation revealed in DCA solvent. The characteristic ratio $\langle R_0^2 \rangle / nl^2$ is obtained from K value estimated from Stockmayer-Fixman plot:

$$[\eta]/M_w^{1/2} = K + (3/2\pi)^{3/2} C\Phi_0 B M_w^{1/2}$$
$$\Phi_0 = 2.5 \times 10^{21}$$

By taking into account the data of Fujita *et al.*[19] on M_w/M_n for poly-γ-benzyl-L-aspertate polymerized with TEA, we assume that the molecular weight distribution of our sample is given by the most probable distribution. The K value obtained from Fig. 20 was introduced into Flory's equation,

$$\frac{\langle R_0^2 \rangle}{nl^2} = \left(\frac{K}{\Phi_0}\right)^{2/3} \frac{M_0}{l}$$
$$M_0 = 143, \quad l = 3.8 \times 10^{-8} \text{ cm}$$

Table 5.

Polymer	Initiator	Solvent	$\langle R_0^2 \rangle / nl^2$	p	Author
PMDLG	TEA	DO/MC	3.72	0.55	
PBDLG	TEA	DO	4.76	0.68	Spach
PDLA	H_2O ?	Acetonitrile	5.4	0.75	Takahashi *et al.*

to obtain the characteristic ratio.

Fig. 16 serves to estimate the sequence probability p, *i.e.*, the isotacticity, yielding $p=0.55$ (Table 5). This value suggests that the equimolar-D,L-copolypeptide used here is nearly randomly sequenced copolymer. Spach[20] has reported $[\eta]$ in DCA and M_w for equimolar-D,L-poly-γ-benzyl glutamate polymerized in dioxane with same initiator as ours. With his data, we arrived at $p=0.68$, a somewhat higher isotacticity. The initiator TEA is an aprotic base and may contribute to produce rather randomly sequenced chains. Nature of solvent used for polymerization is also important to affect the chain growth. Dioxane-methylene chloride 1 : 1 mixture is rather poor solvent for PMDLG, while dioxane is good solvent for PBDLG. In Table 5, the data for equimolar-D,L-polyalanine reported by Takahashi *et al.*[21] Though the type of initiator is not obvious, a large p value ($p=0.75$) estimated should be closely related with the kinds of both initiator and solvent.

Finally we will deal with non-coil conformation of poly-γ-methyl-D,L-glutamates with different L/(L+D) mole% polymerized with TEA as the initiator.

Masuda *et al.*[22] have pointed out that the infra-red peak intensity of the amide V band at 630 cm^{-1} relative to the 2950 cm^{-1} band gives the total helix content, *i.e.*, the sum of right-handed and left-handed helix contents, of solid film. On the other hand, the parameter b_0 of Moffitt-Yang's equation for D,L-copolypeptides in helicogenic solvent may give the difference in right-handed and left-handed helix contents. Thus we can estimate the contents of right-handed helix, left-handed-helix, and random coil, separately. Table 6 shows the samples used for the experiments. The polymer composition represented by L, was determined from the effective residue rotation $[m']$ for copolymers dissolved in DCA, a coil solvent, from $L\% = 100([m']-[m']_D)/([m']_L-[m']_D)$, where subscript D and L denote corresponding homopolymers. It is pointed out that the L%'s in copolymers are slightly higher than the L%'s in the NCA mixture used for polymerization. The total helix contents for solid films casted from chloroform-trifluoro acetic acid (TFA) 95 : 5 mixture, a helicogenic solvent, are compared with those for films casted from TFA, a coil solvent. We may conclude that the helix content is not affected by the type of solvent from which films are casted.

Table 6. Helix Contents of Solid Films of PMDLG

Sample	Monomer Ratio L %	Polymer Compos. [a] L %	Helix-Content % [b] sum	
			Helix-solv.	Coil-solv.
A1	100	100	100	100
A2	90	90.9	96	
A3	80	80.6	84	86
A4	69.9	69.3	76	
A5	60	60.1	64	
A6	50	50.6	60	62
C18	50	50.4		

a) L%=100 $([m']-[m']_D)/([m']_L-[m']_D)$, in DCA
b) Helix content: from $I_{620-630cm^{-1}}/I_{2950cm^{-1}}$, (IR)
c) Helix-solvent: $CHCl_3$-TFA(95:5), film casted therefrom
d) Coil-solvent: TFA, film casted therefrom.

In Fig. 21, the Moffitt-Yang's parameter b_0 was plotted against copolymer composition. The b_0 value for an 80 : 20 blend of L-homopolymer and D-homopolymer fell on the straight line connecting b_0's for both homopolymers. In the figure, the results of Masuda *et al.* for D,L-copolymethyl glutamate polymerized with sodium methoxide in dioxane were compared. Sodium methoxide is an aprotic initiator and in this sense it may be regarded as the same type initiator as our TEA. So their lower b_0 values, *i.e.*, the larger difference in right-handed and left-handed helix contents, may

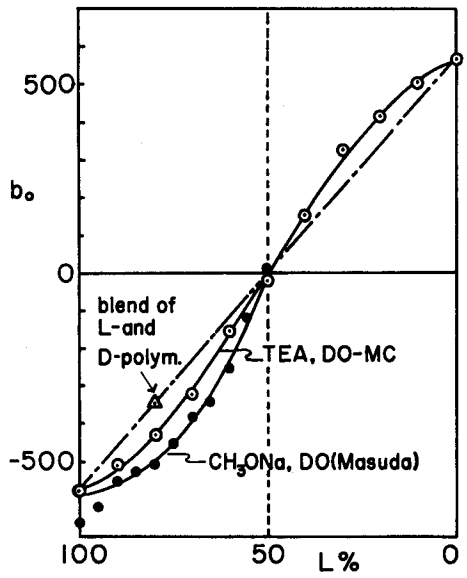


Fig. 21. Moffitt-Yang's b_0 parameter in helicogenic solvent for PMDLG.

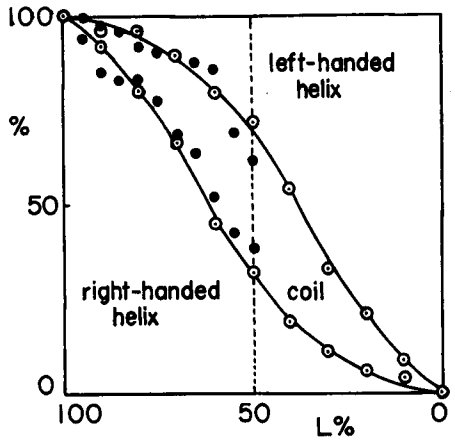


Fig. 22. Contents of right-handed helix, left-handed helix, and random coil in PMDLG. (● Masuda's data).

attributed to solvent effect; dioxane is a better solvent than dioxane-methylene chloride 1 : 1 mixture.

Finally we come to Fig. 22 which shows the contents of right-handed helix, left-handed helix, and random coil as funclions of copolymer composition. For equimolar-D,L-copolymer (L=50%), the ratio, l-helix: r-helix: random coil, is about 30 : 30 : 40 in our case; that of Masuda is about 37.5 : 37 .5 : 25. Though the helix content (sum) estimated from IR is somewhat overestimated because of the overlap of the amide V band of helical form and of the diffuse band of the disordered form, the presence of considerable amount of helical content indicates that the D-residues (L-residues) are appreciably incorporated in the right-handed (left-handed) helical chains composed of L-residues (D-residues).

Doty and Lundberg[23] suggested that the helical sense of a few D-residues added to the right-handed helix composed of L-residues is right-handed, but the addition of more than four D-residues may change the sense of helix. Go and Saito[24] have supported this opinion.

Our experimental result on random chain conformation of equimolar-D, L-poly-γ-methyl glutamate is reproduced as:

characteristic ratio: 3.75

sequence probability $p(=p_{LL}=p_{DD})=0.55$.

For this polymer, *a priori* probabilities p_L and p_D, respectively for L and D, are equal to monomer frequencies 10^{-2}L% and 10^{-2}D%, and $p_L=p_D=0.5$. The dimer frequencies F_{LL} and F_{LD}, *etc.* are related to sequencial probabilities by $F_{LL}=p_L p_{LL}$,

$F_{LD}=p_L p_{LD}$, *etc.*, and $F_{LL}=F_{DD}=0.5\times0.55=0.275$ and $F_{LD}=F_{DL}=0.5\times0.45=0.225$ for this system. Therefore, the frequency of the tetramer LLLL is $0.5\times(0.55)^3$, for example. The obtained value of sequence probability suggests that the stereoregularity of the polymer is slightly isotactic but not much. We may conclude that polymerization, in the present system, proceeds by slightly stereoregular fashion, but not largely deviated from random copolymerization mechanism. Polymer chains formed by such mechanism take also such chain conformation as helix and coil portions occuring alternately along the chain, in helicogenic solvent. The result obtained here is considerably different from the model on polymerization mechanism and chain conformation proposed by Tsuboi *et al.*[25] for D,L-poly-γ-benzyl glutamate prepared in dioxane with sodium methoxide as the initiator.

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References

1) W. G. Miller, D. A. Brant and P. J. Flory: J. Mol. Biol., **23**, 67 (1967).
2) P. R. Schimmel and P. J. Flory: *ibid.*, **34**, 105 (1968).
3) M. Goodman and M. Fried: J. Am. Chem. Soc., **89**, 1264 (1967).
4) F. A. Bovey, J. J. Ryan and F. P. Hood: Macromol., **1**, 305 (1968).
5) D. A. Brant and P. J. Flory: J. Am. Chem. Soc., **87**, 2791 (1965).
6) R. A. Scott and H. A. Scheraga: J. Chem. Phys., **45**, 2091 (1966).
7) L. Pauling: "The Nature of Chemical Bonds," 3rd ed. Cornell Univ. Press, Ithaca, N. Y. (1960).
8) T. Ooi, R. A. Scott, G. van der Kooi and H. A. Scheraga: J. Chem. Phys., **46**, 4410 (1967).
9) J. F. Yan, F. A. Momany, R. Hoffman and H. A. Scheraga: J. Phys. Chem., **74**, 420 (1970).
10) D. A. Brant, W. G. Miller and P. J. Flory: J. Mol. Biol., **23**, 47 (1967).
11) J. E. Mark and M. Goodman: J. Am. Chem. Soc., **89**, 1267 (1967).
12) J. E. Mark and M. Goodman: Biopolymers, **5**, 809 (1967).
13) A. M. Liquori and P. PeSantis: *ibid.*, **5**, 815 (1967).
14) J. H. Fessler and A. G. Ogston: Trans. Faraday Soc., **47**, 667 (1951).
15) S. Tanaka and A. Nakajima: paper presented before the 20th Annual Meeting of the Soc. Polymer Sci. Japan (May 27, 1971): to be submitted to Polymer Journal.
16) S. Tanaka and A. Nakajima: Polymer J., in press (1971).
17) Y. Abe and P. J. Flory: Macromol., **4**, 219, 230 (1971).
18) K. Nagai: J. Chem. Phys., **34**, 887 (1961).
19) Y. Hayashi, A. Teramoto, A. Kawahara and H. Fujita: Biopolymers, **8**, 403 (1969).
20) M. G. Spach: Compt. rend. (Paris), **249**, 543 (1959).
21) A. Takahashi, L. Mandelkern and R. E. Glick: Preprint of the 17th Annual Meeting of the Soc. Polymer Sci. Japan, p. 474, 1968.
22) Y. Masuda, T. Miyazawa and M. Goodman: Biopolymers, **8**, 515 (1969).
23) P. Doty and R. D. Lundberg: J. Am. Chem. Soc., **79**, 2338 (1957).
24) M. Go and N. Saito: J. Phys. Soc. Japan, **20**, 1691 (1965).
25) M. Tsuboi, Y. Mitsui, A. Wada, T. Miyazawa and N. Nagashima: Biopolymers, **1**, 297 (1963).