Chain Conformation of D,L-Copoly(7-methyl glutamate)

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ABSTRACT: The characteristic ratio of equimolar-D,L-poly(γ -methyl glutamate) has been determined from measurements of intrinsic viscosity in good solvent and of molecular weight. Further, the sequence probability p that a residue is succeeded by the same kind of residue was estimated with the aid of the theoretical relation between the characteristic ratio and p reported by us. The obtained result, p=0.55, suggests that L and D residues are approximately randomly-sequenced in the chains. It was also pointed out that the kind of both initiator and solvent in polymerization system closely relates to chain configurations.

KEY WORDS Polypeptide / D,L-Copoly(γ-methyl glutamate) / Chain Conformation / Chain Configuration / Characteristic Ratio / Osmotic Pressure /

A characteristic of synthetic polypeptides is that they are able to form helical conformation, by intramolecular hydrogen bonds, in accordance with circumstance in which molecules are entrusted. The helical conformation is alternatively converted into random coil conformation under a situation in which all the intramolecular hydrogen bonds are broken. With respect to the coil conformation of polypeptides, the same treatment as that for usual linear polymers is possible concerning, for example, characteristic ratio and solution properties. However, for polypeptides, Θ condition is rarely realized, because as the Θ point is approached the molecular chains may transform to helix from coil, or form precipitates. It is difficult to obtain the characteristic ratio from direct measurement at Θ point. Accordingly, the characteristic ratio should be estimated from experimental data measured in good solvent by the aid of a theoretical equation. Experimental values¹ of the characteristic ratio thus obtained for several polyalanine-type homopolypeptides were almost the same, and these values were sufficiently supported by calculated results reported by Flory² and by us.³

Recently, D,L-copolypeptides have attracted attention in various aspects such as polymeri-

zation kinetics, chain conformations, molecular properties, etc. Molecular configuration in terms of stereoregularity should be discussed in connection with the polymerization mechanism, for which experimental accumulation is still lacking. Chain conformation of D,L-polypeptides is investigated on the basis of molecular configuration. Miller, Brant, and Flory,⁴ and the present authors⁵ have reported a theoretical relation between the characteristic ratio and stereoregularity for some D,L-polypeptides, and predicted that the characteristic ratio is remarkably affected by sequence distribution of D or L units in the chain.

The purpose of this work is to determine the characteristic ratio from measurements in good solvent and estimate the sequence distribution of D or L units in the chain, for equimolar-D,L-poly(γ -methyl glutamate).

EXPERIMENTAL

Synthesis of Equimolar-D,L-poly(γ *-methyl glutamate*)

N-Carboxy anhydrides (NCA's) of γ -methyl-Dglutamate and γ -methyl-L-glutamate were separately prepared by introducing phosgene gas into suspensions of γ -methyl-D-glutamate (MDG) or of γ -methyl-L-glutamate (MLG) in tetrahydrofuran (THF). These NCA's were purified by repeated recrystallization from an ethyl acetate

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Sample	NCA, gª	Solvent, ^b m <i>l</i>	Concn of NCA, g/100 ml	[M]° [I]	Yield, %
I	4.060	70	5.8	50	66
Π	3.968	70	5.7	100	88
III	4.029	70	5.8	200	90
IV	4.244	70	6.1	250	90
v	4.022	70	5.8	300	91
VI	4.996	80	7.1	500	98

Table I. Polymerization conditions and per cent polymerization for equimolar-D,L-poly(γ-methyl glutamate)

^a D-NCA+L-NCA (1:1).

^b Dioxane+methylene chloride (1:1).

^e [M], mole concentration of NCA; [I], mole concentration of triethylamine.

solution with the addition of petroleum ether. Equimolar amounts of MLG-NCA and MDG-NCA were then dissolved in a 1: 1-(v/v) mixture of dry dioxane and ethylene dichloride, and polymerized at 25°C in the presence of triethylamine (TEA) as an initiator. The anhydride initiator molar ratio, [M]/[I], used in the experiment are from 50 to 500 to obtain samples of different molecular weights. After polymerization for two weeks at 25°C, D,L-polypeptides formed were precipitated in a large amount of cold methanol, filtered and then dried under reduced pressure. Polymerization conditions and per cent polymerization are summarized in Table I.

Viscosity Measurements

Viscosity measurements were carried out in dichloroacetic acid (DCA) and in *m*-cresol at 25° C with Ubbelohde-type viscometers. From the plot of viscosity number *vs.* concentration, the intrinsic viscosity was determined.

Osmotic Pressure Measurements

Osmotic pressure measurements were carried out in *m*-cresol at 57.8°C with a Knauer Membrane Osmometer. Semi-permeable membranes used were #400 cellophane membranes manufactured by Tokyo Cellophane Co. The conditioning of membranes was performed in eight steps; they were conditioned first in isopropanol and at last in *m*-cresol via toluene. The numberaverage molecular weight M_n and the second virial coefficient A_2 were determined from the plot of $(\pi/c)^{1/2}$ against the concentration c.

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RESULTS AND DISCUSSION

The intrinsic viscosity $[\eta]$, the number-average molecular weight M_n and the second virial coefficient A_2 are summarized in Table II.

Since the polymerization of all the samples is carried out in the same solvent, with the same initiator, and at the same temperature, the polymerization mechanism also can be regarded as identical. Thus, intrinsic viscosities $[\eta]$ (dl/g) in DCA and in *m*-cresol were plotted against the number-average molecular weight on log—log scale in Figure 1. Straight lines in Figure 1 lead to the following equations.

 $[\eta] = 5.9 \times 10^{-5} M_n^{0.85}$ in DCA at 25°C (1)

$$[\eta] = 1.1 \times 10^{-4} M_n^{0.78}$$
 in *m*-cresol at 25°C (2)
32000 < M_n < 82000



Sample	[η] in DCA	[η] in m-cresol	$M_n imes 10^{-4}$	$A_2 imes 10^4$, m l mol/g ²
Ι	0.415	0.348	3.27	1.23
II	0.463	0.424	3.94	1.28
III	0.549	0.456	4.72	1.65
IV	0.614	0.509	5.00	1.32
v	0.634	0.555	5.13	1.31
VI	0.912	0.730	8.21	1.13



Figure 1. Intrinsic viscosity $[\gamma]$ in dichloroacetic acid (DCA) and in *m*-cresol plotted against numberaverage molecular weight M_n , on double logarithmic scale, for equimolar-D, L-poly(γ -methyl glutamate).

To be noticed here is the viscosity behavior of D,L-poly(γ -methyl glutamate) in *m*-cresol. m-Cresol is known⁶ as a helicogenic solvent for $poly(\gamma-methyl-L-glutamate)$. If polypeptide molecules disperse in the form of helix in solution, then the index of the power on M is expected^{7,8} to take a value close to 2. The experimentally obtained value (0.78) of the power for equimolar-D,L-poly(γ -methyl glutamate) is lower than unity and, moreover, the curve for m-cresol system shown in Figure 1 locates below the curve for DCA system, and the power value is lower in *m*-cresol than in DCA. These results mean that the equimolar-D,L-polymer molecules are not dispersed in helical form, even in *m*-cresol which acts as a helicogenic solvent for L- or Dhomopolymer, and that m-cresol behaves as a poorer solvent than DCA which acts as a coil solvent, for the equimolar-D,L-polymer.

The characteristic ratio was estimated in accordance with Stockmayer—Fixman's equation⁹

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

where M_w is the weight-average molecular weight, Φ_0 is a hydrodynamic constant assigned as $\Phi_0 = 2.5 \times 10^{21}$, *B* is a parameter relating excluded volume, and *C* is a constant. This equation was applied to intrinsic viscosity data measured in DCA, a coil solvent. When $[\eta]/M_w^{1/2}$ is plotted against $M_w^{1/2}$, the intercept of straight line on the ordinate gives *K*, from which the characteristic ratio $\langle R_0^2 \rangle / nl^2$ is estimated by the equation

$$\frac{\langle R_0^2 \rangle}{nl^2} = \left(\frac{K}{\Phi_0}\right)^{2/3} \cdot \frac{M_0}{l^2} \tag{4}$$

where, $\langle R_0^2 \rangle$ is the mean square end-to-end length (cm²), l (=3.8×10⁻⁸ cm) is the distance between successive α -carbon atoms, n is the degree of polymerization, and M_0 (=143) is the molecular weight of monomer residue.

For such estimation, the weight-average molecular weight M_w is necessary. Since direct measurements of M_w for the present sample were very difficult, we estimated it from the knowledge in molecular weight distribution. Scoffone, et al.,¹⁰ pointed out by fractionation that $poly(\gamma$ benzyl-L-glutamate) (PBLG) polymerized with either of di-n-butyl amine, di-isopropyl amine or sodium methoxide as an initiator has molecular weight ratio of $M_w/M_n = 1.2 - 1.4$. Fujita, et al.,¹¹ found from measurements of both M_w and M_n that, for PBLG polymerized with trimethylene diamine as an initiator, $M_w/M_n =$ 1.8–2.3. Hayashi, et al., ¹² reported $M_w/M_n = 1.15$ and 1.5 for $poly(\gamma$ -benzyl-L-aspertate) polymerized with triethylamine. With respect to the molecular weight distribution of equimolar-D,Lpolypeptides, nothing is known so far. However, Spach¹³ has reported M_w and $[\eta]$ for equimolar-D,L-poly(γ -benzyl glutamate) polymerized with triethylamine or with sodium methoxide as an initiator. If we assume that the relation between M and $[\eta]$ for monodisperse polymer is the same for both methyl- and benzyl-glutamate equimolar-D,L-polymers, then we have $M_w/M_n=2.1$ by



Figure 2. $[\eta]/M_w^{1/2}$ 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.

combining data obtained by Spach and by ourselves.

The experimental results cited above may allow to assume a molecular weight distribution represented by the most probable distribution for the present polymer. For this type of distribution function, the ratio M_w/M_n is 2.0.

In Figure 2, $[\eta]/M_w^{1/2}$ was plotted against $M_w^{1/2}$, with the use of the weight-average molecular weight thus obtained. Finally, we obtained

$$K = 0.55 \times 10^{-1}$$
$$\frac{\langle R_0^2 \rangle}{m^{1^2}} = 3.72$$

In Figure 2, experimental data for equimolar-D,L-poly(γ -benzyl glutamate) measured by Spach were also plotted.

The curve given in Figure 3 represents the



Figure 3. Characteristic ratio plotted against sequence probability p, for equimolar-D,L-polyalanine-type polymer. Experimental values are denoted by \bigcirc (PMDLG, present work), \bigcirc (PBDLG, Spach¹³), and \triangle (PDLA, Takahashi¹⁴).

theoretical relationship, calculated by us,⁵ between the characteristic ratio and the sequence probability p that a residue is succeeded by the same kind of residue, for equimolar-D,L-polyalanine-type polymer. The experimental value This value $\langle R_0^2 \rangle / nl^2 = 3.72$ leads to p = 0.55. means that the equimolar-D, L-poly(γ -methyl glutamate) (PMDLG) prepared under the conditions mentioned above is nearly randomly sequenced D_{L} -copolymer, though p is slightly larger than 0.5. With the use of the curve in Figure 3, we obtained p also for equimolar-D,L-poly(γ -benzyl glutamate) (PBDLG) reported by Spach (see also Figure 2). His samples were polymerized in dioxane with the same initiator as ours. The sequence probability p (0.68) obtained is somewhat larger than 0.5, but not much larger. These results are shown in Table III. The initiator TEA is an aprotic base and may contribute to produce rather randomly sequenced chains. The nature of solvent used for polymerization is also important to affect the chain growth. Dioxane—methylene chloride-(1:1)mixture is a rather poor solvent for PMDLG, while dioxane is a good solvent for PBDLG. Such difference in solubility may reflect the difference in p value. In Table III, also listed are the data for equimolar-D,L-polyalanine reported by Takahashi, et al.¹⁴ Though the type of initiator¹⁵ is not obvious, a large p value (p=0.75) estimated should be closely related to the kinds of both initiator and solvent.

Investigation such as reported here should be further promoted in connection with researches on polymerization mechanism.

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Initiator $\langle R_0^2 \rangle / n l^2$ Polymer Solvent Author р PMDLG TEA DO/MC^a 3.72 0.55 Present work PBDLG TEA DO 4.76 Spack13 0.68 PDLA Acetonitrile 5.4 0.75 Takahashi, et al.14

Table III. Characteristic ratio and sequence probability for equimolar-D, L-polypeptides

* DO, Dioxane; MC, methylene chloride.

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