Conformational Properties of Poly(γ-methyl-L-glutamate) in Dilute Solution

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From viscosity and osmotic pressure measurements, the characteristic ratio $\langle R_0^2 \rangle / nl^2$ of poly (γ-methyl-L-glutamate) in random coil conformation was estimated. The numerical value obtained supports the theoretical result reported previously.

INTRODUCTION

Recently, theoretical calculations on unperturbed chain dimension have been carried out for random coil chain of polypeptides.[1~6] Conformational properties of polypeptides in dilute solution have also been investigated experimentally.[7~11]

In order to develop the method of conformational analysis toward more complicated molecules such as proteins, and to discuss precisely the thermodynamic properties on helix-coil transition, it is necessary to evaluate the validity of the conformational energy contour map and potential functions for polypeptides.[1~6,12,13] The information on ordered structure of polypeptides in the solid state, obtained from the crystallographic study by X-ray diffraction, contributes only to the potential energy minimum in the contour map calculated theoretically. The characteristic ratio, on the other hand, depends not only upon the location of the potential minimum but also upon the form of potential functions or the shape of the overall surface of conformational energy map. Thus, the measurement of characteristic ratio $\langle R_0^2 \rangle / nl^2$ affords rather exact experimental test on the validity of the theoretical investigation. In this paper, we report experimental results on conformational properties of poly(γ-methyl-L-glutamate)(PMLG) in dilute solution.

PMLG is one of popular synthetic polypeptides at the present stage. However, on account of its relatively poor solubility in solvents and of corrosive nature of solvents in which the polymer is soluble, little has been known about average molecular weights and molecular chain dimensions of the polymer. Further, in *m*-cresol, a helicogenic solvent, for example, the concentration dependence of refractive index is too small to obtain appropriate data from light scattering measurements.

Unavoidably, the choice of the method to determine the chain dimension is restricted. Thus, in the present study, we estimate the characteristic ratio of PMLG by combining the limiting viscosity number in dichloroacetic acid (DCA) with the osmotic pressure data in *m*-cresol.

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EXPERIMENTAL

Materials

γ-Methyl-L-glutamate N-carboxy anhydride (NCA) was prepared according to the method of Blout and Karlson.[14] Poly(γ-methyl-L-glutamate) samples are prepared by polymerizing γ-methyl-L-glutamate NCA under conditions as summarized in Table I.

Solvents used in the preparation of polymer samples and for measurements of viscosity and osmotic pressure were purified in appropriate manners.

Osmotic Pressure Measurement

Stock solutions were filtered and the concentrations were determined by dry weight analysis. Osmotic pressure of polymer solutions was measured by using a Knauer Membrane Osmometer. Gel cellophane No. 400 membranes, supplied by Tokyo

Table I. Summary of Preparative Information on Poly (γ-methyl-L-glutamate) Samples

Sample Code	Solvent	Initiator	$[M]/[I]$[b]
ML311	Nitrobenzene	Diethyl amine	65
ML312	Ethyl acetate	Diethyl amine	32
ML313	Ethyl acetate	Triethyl amine	60
ML310	Nitrobenzene	Triethyl amine	55
ML406	Dioxane-MDC[a]	Triethyl amine	1620
ML306	Dioxane-MDC	Triethyl amine	150

a; Methylene dichloride.
b; Monomer-initiator mole ratio.

Cellophane Co., were used after appropriate conditioning. Measurements were made in m-cresol at 57.8°C. Number-average molecular weight M_n and the second virial coefficient A_2 are obtained from the following equation.

$$(\pi/c)^{1/2}=(RT/M_n)^{1/2}(1+A_2 M_n c/2) \tag{1}$$

where π is the osmotic pressure and c the polymer concentration in g/ml.

Viscosity Measurement

Viscosity measurements were performed in DCA at 25±0.01°C with an improved Ubbelohde viscometer having a flow time for solvent longer than 100 sec.

RESULTS AND DISCUSSION

Experimental results from viscosity and osmotic pressure measurements are summarized in Table II. In Fig. 1, the limiting viscosity number $[\eta]$ (dl/g) is plotted against the number-average molecular weight M_n on double logarithmic scale. A straight line is drawn as to fit in with experimental points. From the straight line in Fig. 1, the coefficients K and a of Mark-Houwink-Sakurada equation $[\eta]=KM^a$ have been determined. The equation obtained is

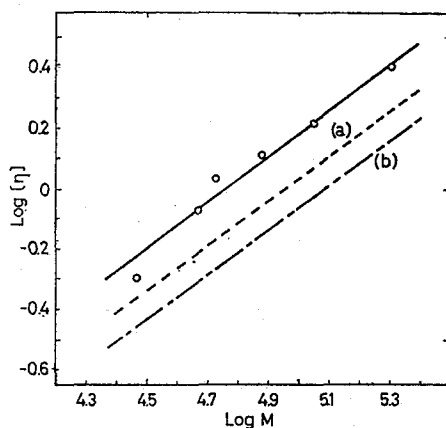$$[\eta]=2.9\times10^{-4}M_n^{0.74}, \text{ in DCA} \tag{2}$$

Fig. 1. Relations between the limiting viscosity number $[\eta]$ (g/dl) in DCA at 25°C and the molecular weight of poly(γ-methyl-L-glutamate) on double logarithmic scale. Open circles represent the experimental points on $[\eta]$ vs. M_n. The broken lines (a) and (b) represent the $[\eta]$ vs. M_w relations obtained from full line by assuming that $M_w/M_n=1.5$ and 2.0, respectively.

Table II. Results of Viscosity and Osmotic Pressure Measurements

Sample Code	$[\eta]$ in DCA (dl/g)	M_n	A_2 (cm^3 mole g^{-2})
ML311	0.51	2.96×10^4	4.0×10^{-4}
ML312	0.86	4.64×10^4	3.5×10^{-4}
ML313	1.09	5.40×10^4	6.2×10^{-4}
ML310	1.31	7.62×10^4	5.5×10^{-4}
ML406	1.67	11.2×10^4	8.1×10^{-4}
ML306	2.58	20.5×10^4	4.8×10^{-4}

The experimental result that $a=0.74$ means that PMLG exists in random coil conformation in DCA. m-Cresol used in the osmotic pressure measurements is known as a helicogenic solvent for PMLG. The second virial coefficient shown in Table II, therefore, is for the molecules in helical conformation.

It was found that polypeptide samples obtained from the polymerization initiated by strong bases had rather narrow molecular weight distribution. For example, it was reported that $M_w/M_n=1.2\sim1.4$ for poly(γ-benzyl-L-glutamate)[15] initiated by either diethyl-, di-n-butyl-, or diisopropyl amine, or by sodium methoxide, and $M_w/M_n=1.15\sim1.5$ for poly(γ-benzyl-L-aspartate)[16] initiated by triehtyl amine (TEA).

Unfortunately, with respect to PMLG, trials to fractionate the samples or to measure the weight-average molecular weight have not been succeeded in. It may not be so absurdity to assume that the molecular weight distribution of the present PMLG is similar to those of polypeptides[15,16] cited above. In this respect, we assumed that $M_w/M_n=1.5$ and that $M_w/M_n=2.0$; the latter corresponds to the "most probable distribution" for the present samples. The broken lines (a) and (b) in Fig. 1 represent the relation between $[\eta]$ and M_w derived from the relation given by Eq. (2) with $M_w/M_n=1.5$ and 2.0,
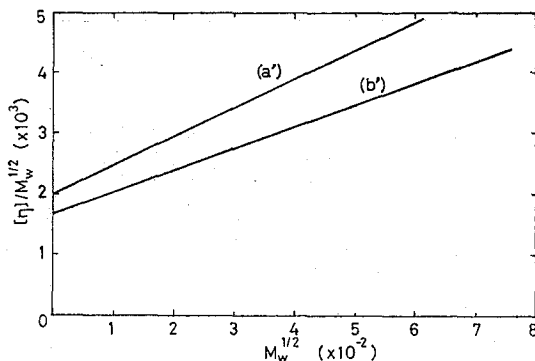
Fig. 2. $[\eta]/M_w^{1/2}$ plotted against $M_w^{1/2}$. Curves (a') and (b') are recast from curves (a) and (b) in Fig. 1, respectively.

respectively.

The straight line relationships given by curves (a) and (b) in Fig. 1, respectively, are recast to the $[\eta]/M_w^{1/2}$ vs. $M_w^{1/2}$ plots, i.e., curves (a') and (b') in Fig. 2, according to Stockmayer-Fixman's relation.[17]

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

From curves (a') and (b'), the values of K are evaluated, and the characteristic ratio $\langle R_0^2 \rangle/nl^2$ is calculated by using the well-known relation,

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

in which $\langle R_0^2 \rangle$ is the mean square unperturbed end-to-end length, n is the number of amino acid residues in a chain, l is the length (3.8 Å) of a virtual bond, M_0 is the weight of an amino acid residue, and $\Phi_0 = 2.5 \times 10^{21}$.

The characteristic ratios obtained were 8.5 and 7.7, respectively, with $M_w/M_n = 1.5$ and 2.0. Taking into consideration of deviation of experimental points from the curve represented by Eq. (2), $\langle R_0^2 \rangle/nl^2$ is estimated as 8.5 ± 0.7 and 7.7 ± 0.8 for $M_w/M_n = 1.5$

Table III. Characteristic Ratios of Polypeptides

Polymer	$[\langle R_0^2 \rangle/nl^2]_{calc.}$	$[\langle R_0^2 \rangle/nl^2]_{obs.}$	Ref.
Poly(γ-methyl-L-glutamate)*		7.7 ± 0.8	this work
Poly(L-alanine)	8.38		5
Poly(γ-benzyl-L-glutamate)		8.8	8
Poly(γ-benzyl-L-glutamate)		7.5 ± 1.2	**
Poly(γ-benzyl-L-aspartate)		9.6	8
Poly(γ-ethyl-L-glutamate)		8.2 ± 0.3	10
Na-poly(L-glutamic acid)		8.8	8
HBr-poly(L-lysine)		8.6	8
Polyglycine	2.17		5
Poly(N-methyl glycine)	2.97	1.8 ± 0.2	11
Poly(N-methyl-L-alanine)	0.58		5

* The observed characteristic ratio is for $M_w/M_n = 2.0$.
** The characteristic ratio was estimated by the authors from the data in ref. 9.

and 2.0, respectively. Both values are not different significantly. In Table III, the characteristic ratio of PMLG obtained above is listed together with the values for various polypeptides observed by other authors[8~11] and the values calculated theoretically in our previous paper.[5] The characteristic ratio obtained in this work is in agreement with the observed values for other α-L-polypeptides having -CH$_2$R side chains and with the calculated value for poly(L-alanine), though some discrepancies are observed among experimental values of characteristic ratio for α-L-polypeptides having different side chains. However, it is difficult to find any systematic trend depending on the kind of side chains.

The experimental evidence presented here appears to support the validity of theoretical treatments on the conformational analysis[1,2,5,6,12,13] for the polypeptide chain, in particular, for poly(L-alanine) chain. Further studies are expected to clarify in detail the effect of side chains on the characteristic ratio for α-L-polypeptides having -CH$_2$R side chains.

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