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Synthesis

Synthesis and Characterization of Three-Branched Star-Like Poly(γ-Benzyl-L-Glutamate)

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SUMMARY

Three branched star-like poly(γ-benzyl-L-glutamate) was synthesized using three functional amine as an initiator, and characterized through light-scattering, ORD and intrinsic viscosity measurements. Experimental results suggest that the synthesized star-like poly(γ-benzyl-L-glutamate)s are composed of three branches of equal length.

INTRODUCTION

As a model protein, solution properties of polypeptides have been studied by many workers. There may be two viewpoints. One is that proteins are copolypeptides consisting of many amino acids. Another is that proteins having S-S bond between cystine residues may be considered as branched polypeptides. From the former viewpoint, many kinds of copolypeptides were synthesized, and informations such as chain dimension, intrinsic viscosity, helix-coil transition parameters were obtained. From the latter viewpoint, comb-like polypeptides have been synthesized in aqueous solution(1-3), and in organic solvents(4,5). The solution properties of comb-like polypeptides have been studied only phenomenologically, and quantitative analysis has not been carried out. This is due to the structural complexity such as the distributions of the degree of polymerization of main chains and branches, and the number of branches. Polypeptides usually take the α-helical conformation in helicogenic solvents such as N,N-dimethylformamide(DMF) and dichloroethane(DCE). So the star-like polypeptides in helicogenic solvent have such a structure that a few polypeptide rods taking α-helical conformation are jointed at the branching point. Three-branched star-like poly(γ-benzyl-L-glutamate)s were synthesized, which were then characterized.

EXPERIMENTAL

Synthesis of Three-Branched Star-Like Poly(γ-benzyl-L-glutamate)

N-Carboxy anhydrides(NCA) of γ-benzyl-L-glutamate(BLG) were prepared (6) by introducing phosgene gas into a suspensions of BLG in tetrahydrofuran(THF). BLG-NCA was purified by repeated reprecipitation from ethyl acetate solution with the addition of petroleum benzine. Then, BLG-NCA was dissolved in a mixture of dry methylene dichloride and dioxane(volume ratio 1:2), and polymerized at room temperature in the presence of diethylenetriamine as initiator([M]/[I]=75). After polymerization for a few weeks at room temperature, polypeptides formed were precipitated in a large amount of methyl alcohol, filtered and then dried under reduced pressure. The product three-branched star-like poly(γ-benzyl-L-glutamate) is abbreviated as PBLG301.

Measurements

Viscosity measurements were carried out in dichloroacetic acid(DCA) at 25°C with a Ubbelohde-type viscometer having flow time for DCA longer than 100 sec. From the plot of viscosity number vs. concentration, the intrinsic viscosity was determined.

FICA light scattering photometer Model 50 was used in an angular range 15° to 150° at 25°C. Vertically polarized light of 546 nm was used. A newly deviced cylindrical cell was used. The constant of the instrument was evaluated with pure benzene as a reference liquid. Coumou's data(7) $R_{Vv}(90)=18.29\times10^{-6}$ and $\rho_u=0.40$ for corresponding depolarization at 25°C for Rayleigh ratio were used. Using benzene for index vat liquid, reflection correction was not necessary, since the refractive index of benzene is very close to that of the cells. Corrections for the effect of the optical anisotropy were made by the measurement at 90° with horizontally polarized light. They were found negligibly small. Solution and solvent were cleaned by centrifugation at a rotor speed 20,000 rpm for 90 min. at room temperature. Refractive index of DMF was 1.428 and refractive index increment($d\tilde{n}/dc$) was 0.118.

A Yanagimoto OR-100 type spectropolarimeter using a tangsten lamp as light source was employed in a temperature range from 20° to 40°C. The wave length used covered from 330 to 590 nm. The concentration of polypeptide solution was about 1.0 gr/dl with all these measurements to cancel the effect of polymer concentration on the optical property.

RESULTS AND DISCUSSION

Figure 1 shows a typical Zimm plots(8,9) of PBLG301 in DMF. In the abscissa $\sin^2(\theta/2)+$(minus constant)$\times c$ was employed instead of the conventional $\sin^2(\theta/2)+$(plus constant)$\times c$ for easy recognition of the slope. Experimental results from light scattering measurement are summerized in Table 1. $\bar{M}_w$ was obtained by the square-root plot, and $\langle S^2\rangle_z^{1/2}$ and A_2 were obtained by Figure 1. The data with $\theta<45°$ in Figure 1 was cut off for the uncertainty caused by strong reduced scattering intensity of solvent in the region of $\theta<45°$.

Moffitt-Yang parameter $-b_o$ was determined by Moffitt-Yang procedure (10) with $\tilde{n}_{DCA}=1.446$. $\tilde{n}_{DCE}=1.444$, $\tilde{n}_{DMF}=1.428$, and M_o(mean residue weight)= 219. Figure 2 shows the temperature dependence of parameter $-b_o$ for PBLG301 in DCA(74 mol%)-DCE(26 mol%) mixture. In Figure 2, Hayashi and Nakajima's results(11) are also indicated for three samples of linear PBLG in DCA(75 mol%)-DCE(25 mol%) mixture. Figure 2 represents the transition temperature dependency on molecular weight. The similar dependency was also reported by Doty and Yang(12), Zimm et al.(13), and Fujita et al. (14). Figure 2 indicates that the behavior of PBLG301 corresponds to that of PBLG having molecular weight less than 4.2×10^4. This result suggests that PBLG301 takes star-like configuration consisting of three-branches. Experimental results on Moffitt parameter $-b_o$ for PBLG301 in DMF are represented in Table 2 together with the results by other workers(11,15). These results also suggest that PBLG301 behaves like a linear PBLG having molecular weight 30,000, that is, PBLG301 is composed of three-branches. The value $-b_o=600$ at 25°C in DMF indicates that PBLG301 takes an α-helical conformation in DMF. Consequently, PBLG301 in DMF is regarded as a helical star-like chain having three rods.

The intrinsic viscosities [η] of PBLG301 in DCA are represented in Table 3. For comparing with the intrinsic viscosity [η] of linear PBLG having the same molecular weight, such polypeptides are needed to prepare. However, synthesis of such sample is very difficult. So, the value calculated from $\bar{M}_w$ employing the relation between the intrinsic viscosity and

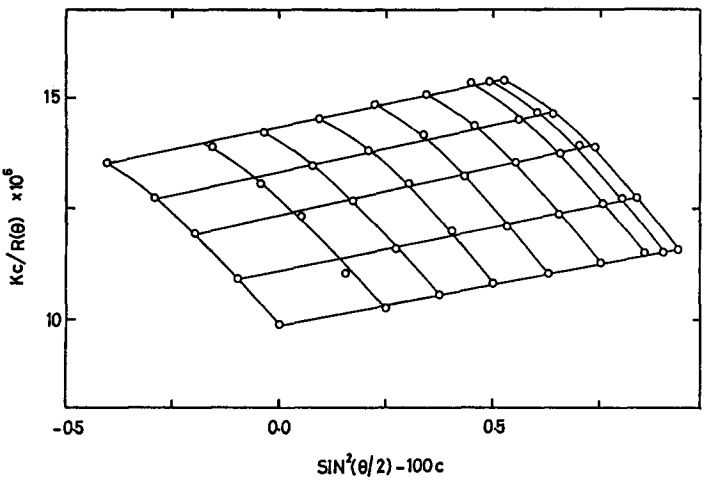

Figure 1. Zimm plot for PBLG301 in DMF at 25°C

Table 1
Results of Light Scattering Measurement

Sample Code	$\bar{M}_w \times 10^{-4}$	$\langle S^2 \rangle_z^{1/2} \times 10^{-2}$ (A)	$A_2 \times 10^4$ (ml²mol/gr)
PBLG301	10.1	2.2_4	5.1_4

molecular weight was used. Norisuye(16) obtained the following Mark-Houwink-Sakurada equation $[\eta]=KM^a$ for PBLG in DCA at 25°C.

$$[\eta] = 1.02\times10^{-4}\ \bar{M}_w^{0.76}$$

Doty et al.(17) also obtained the following equation for PBLG in DCA at 25°C.

$$[\eta] = 2.78\times10^{-5}\ \bar{M}_w^{0.87}$$

Norisuye's equation was obtained with mono-disperse, fractionated PBLG samples, however, Doty's equation was obtained with unfractionated samples. From the dilute solution theory(18,19), the upper limit of the coefficient "a" in the Mark-Houwink-Sakurada equation for random-coil chain is 0.8. But Doty's value(17) exceeds this limiting value. Therefore, we use the Norisuye's equation(16).

Using Norisuye's equation, $[\eta]_{linear}=0.649$ for DCA was obtained. The value of g' given by the ratio of the intrinsic viscosity of branched polymer to that of linear polymer having the same molecular weight, i.e.

$$g' = [\eta]_{branch} / [\eta]_{linear}$$

is g'=0.886 for DCA. In earlier studies on the intrinsic viscosity of branched polymer introduced by assuming the relation

$$[\eta] = k \langle S^2 \rangle_o^{3/2} / M$$

as appeared in Flory-Fox theory(19), g' was given by

$$g' = g^{3/2}$$

where $g = \langle S^2 \rangle_{o,branch} / \langle S^2 \rangle_{o,linear}$

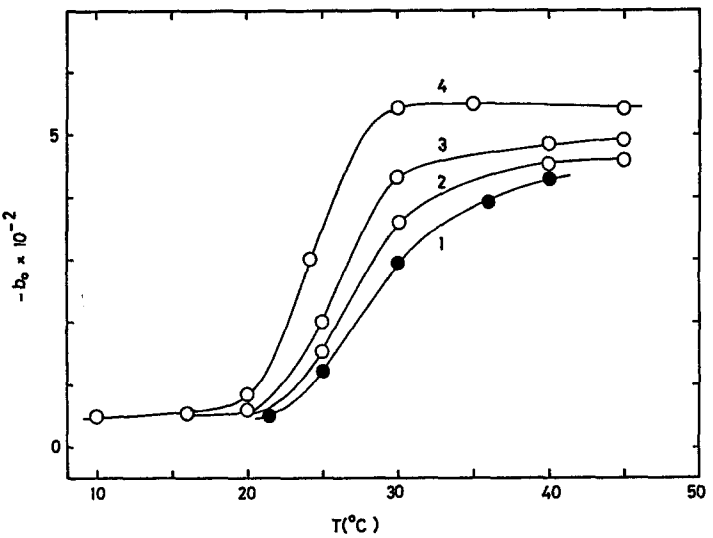

Figure 2. Temperature dependence of $-b_o$ for PBLG301 in DCA(74 mol%)-DCE(26 mol%) mixture and PBLG with various molecular weight in DCA(75 mol%)-DCE(25 mol%) mixture.
1) PBLG301($\bar{M}_w$=10.1×10^4) 2) PBLG-8($\bar{M}_w$= 4.2×10^4)
3) PBLG-7 ($\bar{M}_w$=14.9×10^4) 4) PBLG-3($\bar{M}_w$=45.0×10^4)

Table 2
Moffitt Parameter in DMF

Sample Code	$\bar{M}_w \times 10^{-4}$	$-b_o$(25°C)	$-b_o$(40°C)	Reference
PBLG301	10.1	600	530	This Work
PBLG-3	45.0	630	-	11
PBLG-6	20.0	625	-	11
PBLG-7	14.9	620	-	11
PBLG-12	2.9	595	-	11
A-6	3.7	595	571	15
A-3	1.1	520	518	15

Zimm and Kilb(20) carried out calculation on the intrinsic viscosities of certain branched polymers, taking into account the hydrodynamic interaction between the segments by extending the Zimm theory(21). They found that all calculated results could be approximately given by

$$g' = g^{1/2}$$

and they suggested that this may hold for all the star-like molecules. This rule is good for star-like polymers having not too many branches(22).

The values of g have been calculated by Zimm and Stockmayer(23), and Kurata and Fukatsu(24). Extending the Flory's method(25) for linear polypeptides to branched polypeptides, the values of g for star-like(26) and comb-like(27) polypeptides were calculated as functions of degree of polymerization and number of branches. These results for branched polypeptides showed that the value of g corresponded to the results of Zimm-Stockmayer(23) and Kurata-Fukatsu(24) in the region in which the degree of polymerization is more than 100. The values obtained are g=0.778 for uni-

Table 3
Intrinsic Viscosity of PBLG301 in DCA

Solvent	$[\eta]_{exp}$	$[\eta]^{a}_{linear,calc}$	g'^{b}_{exp}	g'(theory) Uniform	g'(theory) Random
DCA	0.575	0.649	0.886	0.882	0.949

a) Calculated from $\bar{M}_w$ using Norisuye's equation
b) $g'_{exp}=[\eta]_{exp}/[\eta]_{linear,calc}$

form distribution, and g=0.900 for random distribution with three branches. Consequently, g'=0.882 was obtained for uniform distribution and g'=0.949 for random distribution. Experimental result g'=0.886 for ramdom-coil conformation is very close to g' for uniform distribution.

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