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Conformation of Block Copolypeptides of γ-Benzyl L-Glutamate and ε-Carbobenzoxy L-Lysine in *m*-Cresol

Noboru NISHIOKA, Akio TERAMOTO, and Hiroshi FUJITA

Department of Polymer Science, Osaka University, Toyonaka, Osaka 560, Japan.

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ABSTRACT: Block copolypeptides of γ-benzyl L-glutamate (BLG) and ε-carbobenzoxy L-lysine (CBL) were synthesized by polymerization of the corresponding amino acid-*N*-carboxy anhydrides with initiation by *n*-butylamine. Optical rotatory dispersion data showed that the block copolypeptides of the type BLG—CBL—BLG were essentially helical in *m*-cresol at 20°C, despite the fact that in the same solvent conditions isolated CBL chains assume a completely randomly coiled conformation. Dielectric dispersion and viscosity data indicated that the shape of the triblock copolypeptide was rodlike. These results suggest that the central CBL block is forced to take up a rigid helical conformation by an interaction from the flanking BLG blocks, which tend to assume a stable helical conformation under the solvent conditions studied. The conformational induction by the BLG blocks extends over a distance as long as 60 CBL residues when each of the BLG blocks is 20 residues long.

KEY WORDS Block Copolypeptide / γ-Benzyl L-Glutamate / ε-Carbobenzoxy L-Lysine / Polymerization Kinetics / Optical Rotatory Dispersion / Dielectric Dispersion / Dipole Moment / Conformational Induction /

Previous investigations showed that homopolypeptides of γ-benzyl L-glutamyl (BLG) residues dissolved in *m*-cresol assume a stable α-helical conformation,[1-4] while those of ε-carbobenzoxy L-lysyl (CBL) residues in the same solvent undergo a sharp coil-to-helix transition in the temperature region around 25°C.[5-8] The present paper describes an experimental investigation of the question as to whether helix formation of the CBL chain is affected if it is incorporated between blocks of helical BLG chains. The desired triblock copolypeptides with central CBL block of varying length were prepared by successive polymerization of the corresponding amino acid-*N*-carboxy anhydrides with a primary amine as initiator. First, the kinetic processes of the copolymerization were pursued in order to examine whether the procedure used gives samples of the desired copolymer structure. Then, optical rotatory dispersion, dielectric dispersion, and intrinsic viscosity were used to estimate the molecular conformation and shape of the samples in *m*-cresol at room temperature. The results revealed a helix inducing effect of the flanking BLG blocks on the central CBL blocks.

EXPERIMENTAL

Solvents

N,*N*-Dimethylformamide (DMF) was dried with $BaSO_4$ or BaO, and distilled under reduced pressure over BaO, bp 37—40 (8—10 mm). Other organic compounds were thoroughly purified acccording to standard procedures.

Polypeptides

γ-Benzyl L-glutamate-*N*-carboxy anhydride (BLG—NCA) was prepared according to the method of Blout and Karlson.[9] ε,*N*-Carbobenzoxy L-lysine-*N*-carboxy anhydride (CBL—NCA) was prepared from ε,*N*-carbozenzoxy L-lysine according to Fasman, *et al.*,[10] with tetrahydrofuran being used as solvent.

In order to synthesize block copolypeptides of the type BLG—CBL—BLG, the NCA's so prepared were polymerized in DMF at room temperature according to the following scheme. First, BLG—NCA was polymerized with *n*-butylamine as initiator, with the mole ratio of

NCA to initiator fixed at 20. The initial NCA concentration was about 5% by weight. The process of polymerization was followed by measuring the pressure of CO_2 gas evolved. After about three hr the evolution of CO_2 gas stopped, and an aliquot of the polymerization mixture was transferred to a mixture of CBL—NCA and DMF, in which the NCA-initiator mole ratio had been adjusted to a specified value. After the polymerization initiated by the aliquot had been continued for 6 hr, a weighed amount of BLG—NCA dissolved in DMF was added, and the polymerization mixture was allowed to stand overnight with stirring. The final mixture was poured into a large volume of methanol, and the polymer precipitated was collected and freeze-dried from a dioxane solution. In this way, five samples were prepared, which were expected to consist of a CBL block of desired length (5 to 60 residues) sandwiched between two BLG blocks, each consisting of about 20 residues. They were separated into three to five fractions by column elution with methanol—dichloromethane mixtures as eluents, and the central portions of them were used for physical measurements. These fractionation data indicated that the original samples had relatively narrow distributions in both molecular weight and composition. Table I summarizes preparative data.

Table I. Preparative data for block copolypeptides

Sample code	$[A]_0/[I]_0$[a]	$\bar{M}_v \times 10^{-4}$[b]	$\bar{N}_v$	CBL, mol-%[c]
GLG-1	20—5—20	1.0_5	$46._9$	$11._5$
GLG-2	20—10—20	1.1_8	$52._0$	$18._8$
GLG-3	20—20—20	1.3_2	$56._9$	$29._9$
GLG-4	20—40—20	1.7_5	$73._2$	$46._3$
GLG-5	20—60—20	2.3_0	$94._4$	$57._5$
GL-6	20—20	1.1_2	$46._9$	$45._9$

[a] Mole ratio to initiator NCAs.
[b] Calculated from intrinsic viscosities in DCA at 25°C $[\eta]_{DCA}$ using an empirical relation between $[\eta]_{DCA}$ and $\bar{M}_w$ for the block copolymers shown in Figure 6.
[c] Estimated by elemental analysis.

Polymerization Rate

Polymerization rates were determined according to the method of Lundberg and Doty.[11] DMF solutions of NCA and of initiator were mixed in a reaction tube of 2 m*l* capacity, and the amount of CO_2 gas evolved during the course of polymerization was measured conductometrically.[11,12]

Optical Rotatory Dispersion

Optical rotatory dispersions of block copolypeptides in *m*-cresol were measured in the range of wavelength from 300 to 600 nm by use of a JASCO ORD/UV-5 recording spectropolarimeter. The data were analyzed in terms of the Moffitt—Yang equation to determine values of the Moffitt parameters a_0 and b_0, with λ_0 taken to be 212 nm.

Dielectric Dispersion

Dielectric dispersion measurements on *m*-cresol solutions of the fractionated samples in the concentration range 0.4—0.5% were performed over the range of frequency between 250 Hz and 2 MHz by the technique described in our previous papers.[4,7] Extrapolation to infinite dilution was not effected, because our previous experience[4,7] suggested that no concentration effect on dielectric properties would exist in the molecular weight range treated here.

Intrinsic Viscosity and Molecular Weight

Intrinsic viscosities were determined with dichloroacetic acid (DCA) of 25°C and *m*-cresol as solvents. Weight-average molecular weights $\bar{M}_w$ of fractionated samples were determined by the sedimentation equilibrium method[13] with DMF of 25°C as solvent. The partial specific volumes of the samples were estimated from those of pure poly(γ-benzyl L-glutamate) (PBLG) and poly(ε-carbobenzoxy L-lysine) (PCBL), *i.e.*, 0.786 m*l*/g[14,15] and 0.803 m*l*/g,[5] under the assumption that they were linearly related to the weight

Table II. Molecular weights of fractionated block copolypeptides

Sample code	$[\eta]$,[a] dl/g	$\bar{M}_w \times 10^{-4}$	$A_2 \times 10^4$,[b] mol m*l*/g^2	CBL, mol-%[c]
GLG-12	0.158	1.3_1	$8._1$	$11._3$
GLG-22	0.164	1.3_9	$6._9$	$18._8$
GLG-331	0.195	1.6_4	$6._3$	$28._5$
GLG-42	0.198	1.7_9	$6._2$	$45._9$
GLG-52	0.220	2.1_6	$5._0$	$58._9$
GL-6	0.151	1.3_4	$6._7$	$45._9$

[a] Determined in DCA at 25°C.
[b] Second virial coefficients.
[c] Estimated by elemental analysis.

fraction of BLG. Fragmental data for triblock copolypeptides supported this assumption. The results from the molecular weight determination are given in Table II.

RESULTS AND DISCUSSION

Polymerization

Polymerization of NCA initiated with a primary amine usually follows a first-order reaction scheme represented by[9,16-19]

$$\log([A]_0/[A]_t)=0.434[I]_0k_2t \qquad (1)$$

where $[A]_0$ and $[I]_0$ are the molar concentrations of NCA and initiator at time $t=0$, $[A]_t$ is the molar concentration of NCA at time t, and k_2 is the rate constant for the propagation step. Equation 1 holds when initiation step is much faster than the propagation step, and moreover, the latter proceeds without termination. If such an ideal reaction mechanism is obeyed, the product should have a narrow molecular weight distribution represented by Poisson's formula, and the number-average degree of polymerization should be equal to $[A]_0/[I]_0$. In fact, it was found[11,12,16] that polymerization of BLG—NCA in DMF typically obeyed this ideal reaction mechanism, and the finding was successfully used to synthesize once-broken rod polypeptides[4] and DL block copolypeptides of narrow molecular weight distribution.[12] These studies suggest that use of DMF as solvent together with a primary amine as initiator was crucial for realization of the ideal reaction mechanism; polymerization of BLG—NCA in dioxane was shown to follow a two-stage reaction mechanism[11,16] and to yield a very broad distribution of molecular weights. Therefore, the present copolymerization was also carried out with DMF as solvent and *n*-butylamine as initiator, with the expectation that we should obtain samples having a sequence length distribution close to that computed from the $[A]_0/[I]_0$ values taken at the three steps of the copolymerization. The following data confirm this expectation.

Figure 1 illustrates typical kinetic data for the case in which $[A]_0/[I]_0=10$ at each of the three stages. It is to be observed that CO_2 gas started evolving immediately after addition of each NCA solution. The three portions of the curve, two

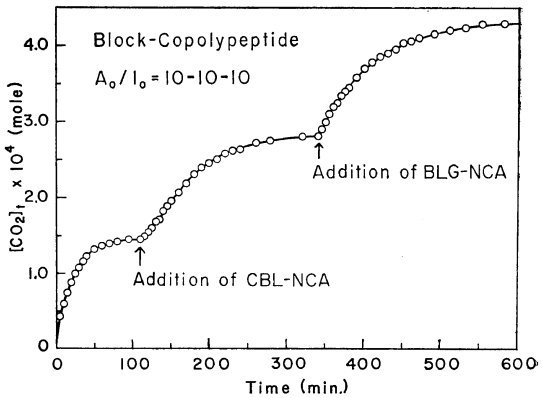

Figure 1. Kinetic curve for block copolymerization of BLG—NCA and CBL—NCA with initiation by *n*-butylamine in DMF at 25°C. The $[I]_0$ at the three stages were 13.3×10^{-3}, 6.63×10^{-3}, and 4.63×10^{-3} mol/*l* respectively, with the mole ratios of NCA being fixed at 10.

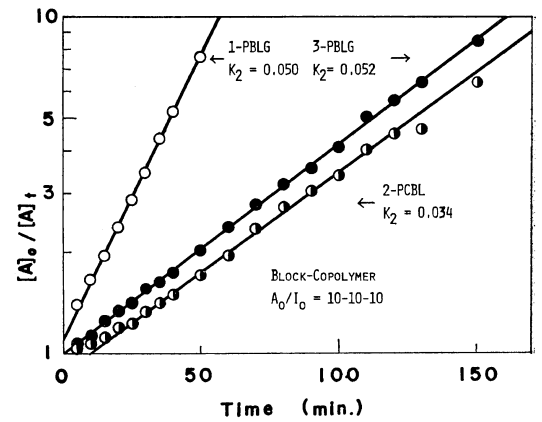

Figure 2. Logarithm of $[A]_0/[A]_t$ plotted *vs.* time with the data shown in Figure 1.

Table III. Polymerization rates of NCAs in DMF at 25°C

NCA[a]	$[A]_0/[I]_0$	k_2, *l*/mol sec
BLG—CBL—BLG	10—5—10	0.053—0.038—0.048
BLG—CBL—BLG	10—10—10	0.050—0.034—0.052
CBL	10	0.039
CBL	20	0.049
CBL	30	0.044

[a] Polymerized with initiation by *n*-butylamine from left to right in the indicated order.

for polymerization of BLG—NCA and one for polymerization of CBL—NCA, are separately replotted in Figure 2 according to eq 1. For all the three kinetic processes the experimental data are seen to obey eq 1. The polymerization rates for this and another case in which $[A]_0/[I]_0=5$ are given in Table III. For comparison, homopolymerization of CBL—NCA was carried out in DMF at 25°C with *n*-butylamine as initiator. The results also followed the first-order reaction mechanism and yielded the rate constants given in Table III. Within experimental uncertainty, the rate constants for CBL—NCA are identical both in the homopolymerization and in the copolymerization. It is also seen that the rate constants for the BLG—NCA in the initial and final steps of the copolymerization are in substantial agreement with each other. Therefore, a block copolypeptide synthesized in this way is expected to have a sequence length distribution corresponding to the amounts of NCAs fed successively.

Preparative polymerizations were carried out in a similar fashion, expect that reaction times were longer than those for the kinetic experiments. The results are summarized in Table I, which, as expected from the above-mentioned discussion of the kinetic data, shows that the viscosity-average degrees of polymerization $\bar{N}_v$ of the samples obtained are in good agreement with the total mole ratios of NCA to initiator. The mole fractions of CBL residues determined from elemental analysis also agree with those calculated from the amounts of the NCAs used.

Optical Rotatory Dispersion

Table IV lists values of the Moffitt parameters a_0 and b_0 obtained. The values of b_0 for the triblock copolypeptides are in the range between −460 and −540 at −20°C and a little larger at 40°C. With the understanding that b_0 is a direct measure of helical fraction, we may conclude for the reason given later that the triblock copolypeptides examined are essentially helical in *m*-cresol.

As the degree of polymerization N decreases, the b_0 of PBLG in *m*-cresol shows a systematic increase, starting from a value of −630 characteristic of α-helix at very large N and reaching −540 at $N=50$ when estimated from available data.[3,12,20] No direct estimation of the b_0 for helical PCBL in *m*-cresol is feasible, because this polypeptide in *m*-cresol cannot be brought to a perfect helix in the temperature range accessible to experiment. On the other hand, both PBLG and PCBL become helical in DMF, but the $-b_0$ value of the latter is lower than that of the former by 10—15%.[3] If a similar difference is assumed to exist for *m*-cresol solutions, we may assign a value of −460 for the b_0 of a helical PCBL sample with an N of 50 in *m*-cresol. Thus the b_0 values for helical triblock copolypeptides with an N of 50 should increase from −540 to −460 as the CBL content increases.

Table IV. Moffitt parameters for block copolypeptides in *m*-cresol

Sample code	$-b_0$		a_0	
	20°C	40°C	20°C	40°C
GLG-12	53_9	51_7	36_2	33_3
GLG-22	53_2	51_3	32_3	29_4
GLG-331	51_3	49_3	26_7	24_3
GLG-42	45_9	44_3	15_8	14_6
GLG-52	46_3	46_6	11_1	11_9
GL-6	40_5	43_6	15_8	17_1

Looking at Table IV with this expectation, one finds that the triblock copolypeptides studied all assume helical conformations in *m*-cresol at 20 and 40°C. Since isolated PCBL chains in the same solvent at 20°C are randomly coiled, this finding implies that the flanking helical BLG blocks act to induce helical winding of the central CBL block. Naturally, the fact that both PBLG and PCBL form helices of the same screw sense (right-handed) should favor the occurrence of such an induction effect, but it is rather surprising that the effect persists even in the sample GLG-52 which contains as long a central CBL block as 60 residues.

The b_0 for a BLG—CBL diblock copolymer GL-6 is −405 at 20°C and decreases to −436 at 40°C. This fact indicates that the CBL block, which is partially helical even at 20°C presumably due to conformational induction of the BLG block, becomes more helical at 40°C, and it is consistent with the observation that PCBL itself undergoes a thermally-induced coil-

to-helix transition in the temperature range 20—40°C.[5-8]

Conformational induction has also been reported for DL and other types of block copolypeptides,[11,21,22] and the findings have been used to determine the unknown screw sense of particular residues blocked with residues of a known screw sense.[21,22] The extent of conformational induction is expected to depend on the sequence distribution of blocks as well as on the relative stability of their helices, *i.e.*, the free energy difference between helical residues. Some theoretical calculations of this dependence have been reported,[22-24] but the present data are not amenable for a direct comparison with them.

As an extreme case one may consider a triblock copolypeptide in which the helix-forming ability of the flanking A blocks is so great that their terminal residues adjacent to the central B block always maintain a helical conformation. Theoretical calculations with this model show that even in a solvent in which an isolated B block favors random coil conformation, there is induced helical winding of the central B block. Here one finds a possibility of explaining the conformational induction observed in the present work. In order to make it conclusive, however, one must determine, among others, the free energy difference between helical BLG and CBL in *m*-cresol. In this connection we wish to remark that Klug and Applequist[22] have recently analyzed ORD data for a series of DL block copoly(γ-benzyl glutamate)s of varying DL ratios. They obtained a value of 100—400 cal/mol for the free energy difference between right- and left-handed α-helices of PBLG and of poly(L-tyrosine) and compared it with an estimate from conformational analysis.[25]

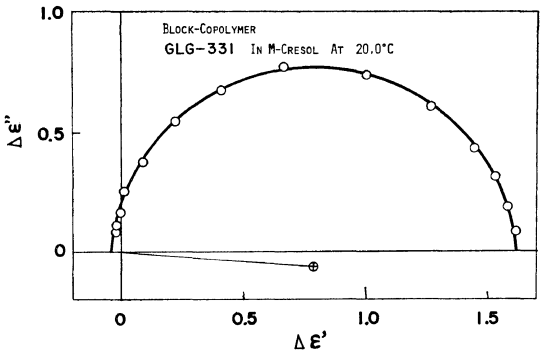

Figure 3. Cole—Cole plot for sample GLG-331 in *m*-cresol at 20°C. $c=4.660\times10^{-3}$ g/m*l*.

Dielectric Dispersion

Dielectric dispersion curves obtained on *m*-cresol solutions of the block copolypeptides were analyzed to determine mean-square dipole moment $\langle\mu^2\rangle$ and mean rotational relaxation time τ. Figure 3 shows a typical Cole—Cole plot for sampe GLG-331 in *m*-cresol at 20°C. The data points taken in the frequency range between 250 Hz and 2 MHz fall on a semicircle whoes center is located close to the $\Delta\varepsilon'$-axis. Here $\Delta\varepsilon'$ and $\Delta\varepsilon''$ are the real and imaginary parts of the complex dielectric constant of the solution in excess of those of the solvent. The segment cut out of the $\Delta\varepsilon'$-axis by the arc is taken to be the static dielectric increment $\Delta\varepsilon$, and the frequency f_c corresponding to the summit of the arc is related to the mean rotational relaxation time τ by $\tau=1/(2\pi f_c)$. The closeness of the plot to the Debye circle in Figure 3 indicates that the sample is nearly homogeneous with respect to molecular weights, too. Similar results were obtained for other fractionated samples. Unfractionated sample GL-6 gave a

Table V. Dielectric dispersion data for block copolypeptides in *m*-cresol

Sample code	$\langle\mu^2\rangle^{1/2}$, D				$\tau T/\eta_0\times10^3$, sec deg/poise			
	10°C	20°C	30°C	40°C	10°C	20°C	30°C	40°C
GLG-12	28_7	29_5	28_9		3.1_4	3.0_7	2.9_0	
GLG-22	29_9	30_5	31_0		3.5_2	3.3_7	3.1_2	
GLG-331	34_9	35_2	35_9		4.9_1	4.7_6	4.7_4	
GLG-42	37_4	37_9	38_3	37_8	5.6_6	5.5_2	5.2_9	4.9_4
GLG-52		45_7	45_8	45_3		8.2_5	7.6_9	7.4_1
GL-6	22_7	23_4			2.8_9	2.7_9		

Cole—Cole plot whose center displaced a little more downward. This fact suggests that it had a broader molecular weight distribution than those of the fractionated samples. However, from our previous studies with PBLG,[4] we infer that the polydispersity index $\bar{M}_w/\bar{M}_n$ of sample GL-6 would not have been more than 1.2, where $\bar{M}_n$ is the number-average molecular weight.

In order to calculate the mean-square dipole moment $\langle\mu^2\rangle$ from experimental values for $\Delta\varepsilon$, we made use of the equation proposed by Applequist and Mahr,[26] which can be recast at the limit of infinite dilution in the form[4]

$$\langle\mu^2\rangle=\frac{3kTM}{4\pi N_A}(\Delta\varepsilon/c)(q/fg) \qquad (2)$$

where M is the molecular weight of the polypeptide, k is the Boltzmann constant, T is the absolute temperature, N_A is the Avogadro number, and c is the solute concentration in g/m*l*. The factor q/fg depends primarily on the shape of the solute molecule; it is determined in particular by the axial ratio p for ellipsoids of revolution.[4,26-28] We here assume[4,28] that the molecular shape of a copolypeptide is approximated by an ellipsoid of revolution with an axial ratio equal to $\bar{N}_w/10$, with $\bar{N}_w$ being the weight-average degree of polymerization. Table V, which summarizes the calculated results, shows that $\langle\mu^2\rangle^{1/2}$ for the triblock copolypeptides in *m*-cresol at 20°C are approximately proportional to $\bar{N}_w$. This proportionality suggests that the molecular shape is rodlike, a fact compatible with the conclusion from the ORD data that the triblock copolypeptides examined are essentially helical under the same solvent conditions.

Examination of Table V reveals that the average dipole moments per peptide residue $\langle\mu^2\rangle^{1/2}/\bar{N}_w$ for the block copolymers are consistently larger than 4.7 D, the value deduced for PBLG in helicogenic solvents.[4,30] Figure 4 illustrates this trend more clearly, in which $\langle\mu^2\rangle^{1/2}/\bar{N}_w$ is plotted against the mole fraction of CBL residues x_L; the circle at $x_L=0$ denotes the value for PBLG. If the molecular shape is that of a straight rod, the value for $\langle\mu^2\rangle^{1/2}/\bar{N}_w$ should vary linearly with x_L, *i.e.*,

$$\langle\mu^2\rangle^{1/2}/\bar{N}_w=(\mu_L-\mu_G)x_L+\mu_G \qquad (3)$$

where μ_G and μ_L are the monomeric dipole moment along the helices of BLG and CBL residues, respectively. Although the data points do not conform precisely to eq 3, the $\langle\mu^2\rangle^{1/2}/\bar{N}_w$ appears to increase with x_L. An approximate estimation from the indicated line yields 5.6± 0.3 D for μ_L. This value is in fair agreement with 5.4 and 6.2 D deduced for PCBL in *m*-cresol by Omura, *et al.*,[7] from dipole moment data in the helix-coil transition region. Therefore we conclude that the average dipole moment

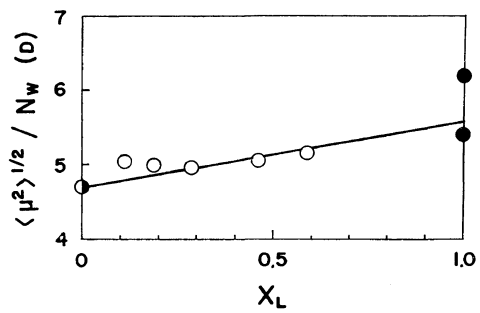

Figure 4. Monomeric dipole moment of BLG—CBL—BLG copolypeptides in *m*-cresol at 20°C as a function of mole fraction of CBL, x_L. The circle at $x_L=0$ denotes the value for PBLG in *m*-cresol[4] and those at $x_L=1$ denote the values for PCBL in *m*-cresol deduced indirectly by Omura, *et al.*[7]

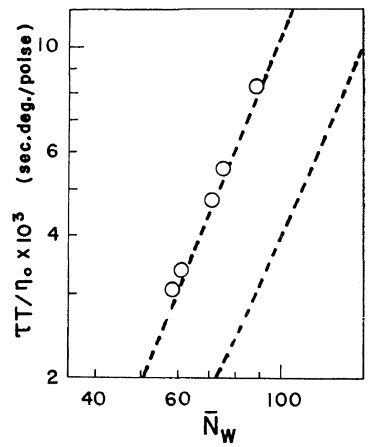

Figure 5. Double logarithmic plot of $\tau T/\eta_0$ *vs.* $\bar{N}_w$ for block copolypeptides of BLG and CBL in *m*-cresol at 20°C. The dashed lines denote the data for straight rod PBLG (upper) and for once-broken rod PBLG (lower), respectively.[4]

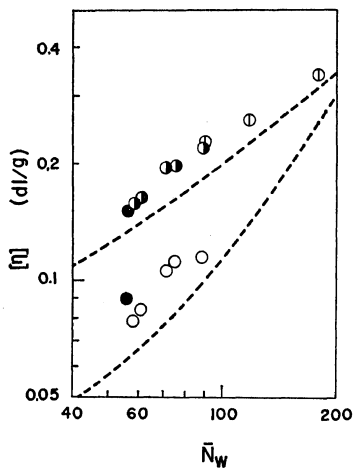

Figure 6. Comparison of intrinsic viscosity $-\bar{N}_w$ relationships. (ⓘ), PCBL in *m*-cresol at 15°C;[7] (○), block copolypeptides in *m*-cresol at 20°C and (◑), in DCA at 25°C; (●), data for sample GL-6. The dashed lines denote the data for PBLG in DCA (upper) and in DMF (lower) at 25°C.[12,28,31]

per helical residue is larger for CBL than for BLG. Much lower values would be obtained for $\langle\mu^2\rangle^{1/2}/\bar{N}_w$ if the central CBL block were randomly coiled and bent. Note that the $\langle\mu^2\rangle^{1/2}/\bar{N}_w$ for the once-broken rod PBLG is nearly half as large as that for the straight rod one.[4,29]

Figure 5 shows double-logarithmically the $\bar{N}_w$ dependence of mean rotational relaxation time τ corrected for solvent viscosity η_0 and absolute temperature T, where the dashed lines denote the data by Matsumoto, *et al.*,[4] for PBLG in helicogenic solvents. The data for the triblock copolymers follow closely the curve for straight rod PBLG and are much larger than those for once-broken rod ones. These results also lend support to the view that the molecular shape of the triblock copolypeptides in *m*-cresol is rod-like.

Intrinsic Viscosity

Intrinsic viscosities in a helix-breaking solvent DCA and in *m*-cresol are compared in Figure 6, where the two dashed lines represent the data for PBLG in DCA and in a helicogenic solvent DMF.[12,28,31] In this range of $\bar{N}_w$, the statistical radius of a polypeptide molecule should be smaller in the helical state than in the random coil state, as is its intrinsic viscosity also.[1,2,28,31] The present data for the triblock copolypeptides are consistent with this prediction, because, as demonstrated by the ORD and dielectric data, the molecular conformation is helical in *m*-cresol and randomly coiled in DCA.

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REFERENCES

1. P. Doty, A. M. Holtzer, J. H. Bradbury, and E. R. Blout, *J. Amer. Chem. Soc.*, **76**, 4493 (1954).
2. P. Doty, J. H. Bradbury, and A. M. Holtzer, *ibid.*, **78**, 947 (1956).
3. T. Matsumoto and A. Teramoto, *Biopolymers*, **13**, 1347 (1974).
4. T. Matsumoto, N. Nishioka, A. Teramoto, and H. Fujita, *Macromolecules*, **7**, 824 (1974).
5. M. Matsuoka, T. Norisuye, A. Teramoto, and H. Fujita, *Biopolymers*, **12**, 1515 (1973).
6. K. Nakamoto, H. Suga, S. Seki, A. Teramoto, T. Norisuye, and H. Fujita, *Macromolecules*, **7**, 784 (1974).
7. I. Omura, A. Teramoto, and H. Fujita, *ibid.*, **8**, 284 (1975).
8. A. Nakajima, T. Hayashi, and S. Emi, Preprint, 17th Polymer Symposium, Kobe (1971).
9. E. R. Blout, and R. H. Karlson, *J. Amer. Chem. Soc.*, **78**, 941 (1956).
10. G. D. Fasman, M. Idelson, and E. R. Blout, *ibid.*, **83**, 709 (1961).
11. R. D. Lundberg and P. Doty, *ibid.*, **79**, 3961 (1957).
12. K. Nakagawa, N. Nishioka, A. Teramoto, and H. Fujita, *Polymer J.*, **3**, 332 (1973).
13. H. Fujita, "Foundations of Ultracentrifugal Analysis," Wiley-Interscience, New York, N.Y., 1975.
14. C. De Loze, P. Saludjian, and A. J. Kovacs, *Biopolymers*, **2**, 43 (1964).
15. T. Norisuye, M. Matsuoka, A. Teramoto, and H. Fujita, *Polymer J.*, **1**, 691 (1970).
16. J. C. Mitchell, A. E. Woodward, and P. Doty, *J. Amer. Chem. Soc.*, **79**, 3955 (1957).
17. M. Idelson and E. R. Blout, *ibid.*, **79**, 3948 (1957).
18. M. Szwarc, *Adv. Polym. Sci.*, **4**, 1 (1965).
19. Y. Imanishi, *Kobunshi* (*High Polymers, Japan*), **21**, 32, 92 (1972).
20. J. Y. Cassim and E. W. Taylor, *Biophys. J.*, **5**, 553 (1965).
21. L. Palillo, P. Temussi, E. Trivellone, E. M. Bradbury, and C. Crane-Robinson, *Biopolymers*,

10, 2555 (1971).
22. T. L. Klug and J. Applequist, *ibid.*, **13**, 1317 (1974).
23. M. Go and N. Saito, *J. Phys. Soc. Japan*, **20**, 1686 (1965).
24. P. H. von Dreele, D. Poland, and H. A. Scheraga, *Macromlecules*, **4**, 396 (1971).
25. T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).
26. J. Applequist and T. G. Mahr, *J. Amer, Chem. Soc.*, **88**, 5419 (1966).
27. A. D. Buckigham, *Aust. J. Chem.*, **6**, 93, 323 (1952).
28. A. Teramoto and H. Fujita, *Adv. Polym. Sci.*, **18**, 65 (1975).
29. H. Kihara, K. Tanno, and A. Wada, *Polymer J.*, **5**, 324 (1973).
30. H. Kihara, *ibid.*, **7**, 406 (1975).
31. T. Norisuye, Ph. D. Thesis, Department of Polymer Science, Osaka University, 1973.