Synthesis and Structure Studies of Copolypeptides Composed of γ -Benzyl-L-Glutamate and γ -Methyl-L-Glutamate

Toshio KANAMORI*, KOZO ITOH, and AKIO NAKAJIMA**

*Department of Polymer Chemistry, Kyoto University,
Sakyo-ku, Kyoto, Japan.*

(Received April, 30, 1970)

ABSTRACT: Copolypeptides composed of γ -benzyl-L-glutamate and γ -methyl-L-glutamate covering the whole range of copolymer compositions were synthesized by the NCA method. The experimentally obtained monomer reactivity ratios, r_1 (for BLG)=0.70 and r_2 (for MLG)=1.40, suggest that the polymers formed are regarded approximately as random copolymers. The infrared spectra of these copolypeptides in solid state indicated that these copolymer molecules can exist in α -helix conformation. The chain packing in unit cells for these polymers was investigated from X-ray diffraction patterns as a function of copolymer composition. The distance between adjacent chains was found to change linearly with copolymer composition.

KEY WORDS Polypeptide / Copolymer of γ -Benzyl-L-Glutamate and γ -Methyl-L-Glutamate / Chain Conformation / Infrared Spectra / X-ray Diffraction / Viscosity /

Copolypeptides composed of two amino acids having inert side chains are known¹ to be prepared from *N*-carboxy- α -amino acid anhydrides (NCA) by essentially the same method as used for homopolypeptides. Also supposed is that a mixture of NCAs will in general lead to a random copolymer, in which the overall composition is governed by the molar ratio of the NCAs used. However, the comonomer arrangement or randomness in comonomer distribution in the chain should practically depend on the relative reaction rates, *i.e.*, the monomer reactivity ratios, of the NCAs used. Thus, if two NCAs having the same order of monomer reactivity ratios are copolymerized, the sequences of residues in the resulting copolymer chain will be randomly distributed.

Triethylamine as an initiator is known² to yield polypeptides with high degrees of polymerization. In this work, copolypeptides of γ -benzyl- and γ -methyl-L-glutamates covering comonomer ratios from 0 to 1 were synthesized by the NCA method initiated by triethylamine. Although analysis in terms of radical addition

kinetics may not be directly applicable to our system, we may say that the conditions under which the polymers were synthesized sensibly satisfy the requirements for using this type of analysis.

With these samples, in the next place, the molecular structure in solid state was analyzed as a function of copolymer composition by means of the infrared spectra and X-ray diffraction patterns.

EXPERIMENTAL

Synthesis of Copolypeptides

The monomers, *N*-carboxy- γ -benzyl-L-glutamate (BLG-NCA) and *N*-carboxy- γ -methyl-L-glutamate (MLG-NCA) were prepared by the method proposed by Blout and Karlson³, and purified by repeated recrystallization from an ethyl acetate solution with the addition of petroleum ether. The BLG-NCA and MLG-NCA, in desired mole ratios, were dissolved in a 1:1 (v/v) mixture of dry dioxane and ethylene dichloride. The total anhydride concentration was kept at 3%. The polymerization was initiated with triethylamine (TEA) at an anhydride: initiator ratio (M/I) of 500, 200, or 50. The poly-

* Recently the family name was changed from Hayashi to Kanamori.

** To whom correspondence should be addressed.

merization was completed within about 50 hr at 25°C. Samples in series A were allowed to polymerize until no further evolution of CO₂ gas was observed, *i.e.*, up to a high conversion level. Samples in series B were stopped polymerizing at a lower conversion stage so as to obtain the monomer reactivity ratios. Samples with different molecular weights were prepared by varying the concentration of NCA and TEA in the system. The copolypeptides formed were precipitated in a large amount of cold methanol and dried under reduced pressure at 50°C.

Compositions of these copolypeptides were determined from the analytical values of C and H. These elementary analyses were carried out in the Organic Microanalysis Center in Kyoto University.

The limiting viscosity numbers $[\eta]$ as a measure of molecular size of these copolypeptides were determined in dichloroacetic acid (DCA) at 25°C with Ubbelohde type viscometers.

The experimentally determined monomer ratio in the copolymer and the initial composition of the monomer mixture, together with the limiting viscosity number, were summarized in Table I, in which series A and B, respectively, correspond

to high and low conversion runs.

Infrared Spectra

Infrared absorption spectra of solid films of the samples cast from chloroform solution were measured with a Perkin-Elmer Model 521 spectrophotometer in the spectral region of 4000–400 cm⁻¹.

X-ray Diffraction

X-ray diffraction diagrams were obtained with solid films mentioned above. Oriented films were also prepared by stroking the films in one direction during drying. X-ray photographs were taken with a flat-plate camera by using nickel-filtered CuK_α radiation.

RESULTS AND DISCUSSION

Randomness in Comonomer Arrangements along the Chain as Inferred from the Monomer Reactivity Ratios

Figure 1 illustrates the copolymer composition curve for the copolymerization of γ -benzyl-L-glutamate (BLG) with γ -methyl-L-glutamate (MLG) at conversion levels about 40%, being taken from the data on series B in Table I. As is obvious from Figure 1, MLG was slightly

Table I. Copolymerization of γ -benzyl-L-glutamate with γ -methyl-L-glutamate by the NCA method

No.	M/I	Initial monomer Ratio, mol%		Polymer composition, mol%		$[\eta]$, dl/g (DCA, 25°C)	Conversion %
		BLG	MLG	BLG	MLG		
A1	200	0.0	100.0	0.0	100.0	2.78	94.7
A2	200	20.0	80.0	19.0	81.0	2.00	96.5
A3	200	30.0	70.0	29.0	71.0	1.72	96.4
A4	50	45.0	55.0	47.1	52.9	0.66	91.0
A5	200	50.0	50.0	52.1	47.9	1.33	96.6
A6	200	70.0	30.0	71.2	28.8	2.02	94.8
A7	200	80.0	20.0	81.1	18.9	2.21	94.3
A8	200	100.0	0.0	100.0	0.0	1.25	95.2
B1	500	0.0	100.0	0.0	100.0	0.410	40.5
B2	500	20.0	80.0	16.5	83.5	0.205	39.9
B3	500	30.0	70.0	23.4	76.6	0.200	43.4
B4	500	50.0	50.0	44.2	55.8	0.050	21.0
B5	500	50.0	50.0	44.8	55.2	0.072	42.5
B6	500	50.0	50.0	45.2	54.8	0.079	59.9
B7	500	70.0	30.0	62.6	37.4	0.120	41.3
B8	500	80.0	20.0	75.0	25.0	0.185	40.1
B9	500	100.0	0.0	100.0	0.0	0.330	42.5

Figure 1. Copolymer composition curve for PBMLG copolymer at ca. 40% conversion. Data are taken from series B in Table I.

Figure 2. Composition vs. conversion curve for 50-mol% BLG—50-mol% MLG system. The points show the data of B4, B5, B6, and A5 in Table I, respectively, from left to right.

more reactive than BLG but the deviation from the linear relationship was very small. In Figure 2 is shown the MLG mol% in copolymer against the per cent polymerization for the systems derived from a 50:50 mol% monomer mixture.

Now we denote the monomer reactivity ratios for the copolymerization of BLG with MLG by r_1 and r_2 , respectively. To obtain the monomer reactivity ratios, in this work, two methods were attempted; one is the method proposed by Mayo and Lewis⁴ which used the integrated form of copolymer equation, and the other is the method

to plot the mole fraction of monomer 1 in the copolymer against the mole fraction of monomer 1 in the monomer feed according to Fineman and Ross.⁵ The numerical values of r_1 and r_2 obtained from the data shown in Table I were as follows

$$\begin{aligned} r_1 &= 0.70 \quad (\text{Fineman-Ross}) \\ &= 0.65 \quad (\text{Mayo-Lewis}) \\ r_2 &= 1.40 \quad (\text{Fineman-Ross}) \\ &= 1.50 \quad (\text{Mayo-Lewis}) \end{aligned}$$

The results by these different methods were in fair agreement with each other.

It should be noticed here that the product $r_1 \times r_2$ was very close to unity, i.e., $r_1 \times r_2 = 0.98$, which means that the type of this copolymerization is almost ideal. In other words, the two monomers BLG and MLG are arranged at random along the chain in relative amounts specified by the composition of the initial monomer mixture. The same conclusion was obtained previously by Nylund⁶ for copolymerization of NCAs of L-leucine and L-glutamic acid.

Verifications that the polymers obtained here are true copolymers will be reported in the next paper by comparing the optical rotatory dispersions on the copolymer and homopolymer blends. Solubility experiments may also afford some information to this end. For example, PMLG does not dissolve in DCE, dioxane, and DMF, while PBMLG's containing BLG residues of more than thirty per cent easily dissolve in these solvents.

Chain Conformation of Random Copolypeptide PBMLG in Solid State

Infrared spectra were measured with solid films of copoly (γ -benzyl- γ -methyl-L-glutamate) (designated as PBMLG) with different copolymer compositions.

Some typical illustrations of these spectra on PBMLG were shown in Figure 3 together with those of homopolymers PBLG and PMLG. As has been pointed out by Miyazawa and Blout^{7,8}, the amide I band, due to C=O stretching vibration, and the amide II band, associated with C=N stretching and N-H deformation, of the α -helical conformation are expected at 1655 and 1550 cm^{-1} , respectively. As is obvious from

Synthesis and Structure Studies of Copolypeptides

Figure 3. Infrared spectra for (a) PBLG [sample A8], (b) PBMLG (BLG, 71 mol%) [sample A6], (c) PBMLG (BLG, 52 mol%) [sample A5], (d) PBMLG (BLG, 29 mol%) [sample A3], and (e) PMLG [sample A1].

Figure 3, the amide I and amide II bands of these copolymers were observed at the same wave numbers as those for both PBLG and PMLG, *i.e.*, at 1665–1655, and 1550 cm^{-1} , respectively. This fact is in conformity with Miyazawa's remark that these bands depend primarily upon the main chain conformation and are not largely affected by the nature of side chains.

The amide V band, assigned to the N—H out-of-plane depending, has been found^{9,10} to be sensitive to the chain conformations even for unoriented samples used to estimate the helix contents. According to the study of Miyazawa, *et al.*⁹, the amide V band of the α -helical form was reported to appear at 610–620 cm^{-1} for PMLG, PBLG, poly (γ -ethyl-L-glutamate), and poly-L-alanine, while that of the disordered form at 650 cm^{-1} . As a matter of course, the amide V band can more preferably be used to estimate helix contents rather than the amide I or II band.

Recently, Shimanouchi, *et al.*¹¹, have studied far-infrared spectra of the α -helical form of some polypeptides and have found that the main chain vibrations in the region from 600 to 200 cm^{-1}

are strongly coupled with the side chain deformation vibrations, but the absorption of the α -helical form appearing at 610–620 cm^{-1} is not affected by the kind of the side chains.

As is obvious in Figure 3, the amide V band for copolymers PBMLG's appeared at 620 cm^{-1} , just the same wave number as for PBLG and PMLG, with almost the same order of peak intensity as that of homopolymers. Such a result means that these PBMLG's exist in helical conformation and moreover the helix contents of these copolymers are nearly the same as those of homopolymers. The intensity of the amide V band at 620 cm^{-1} relative to the 2950 cm^{-1} band

Table II. Infrared intensity ratio ($620\text{ cm}^{-1}/2950\text{ cm}^{-1}$) for PBMLG, PBLG, and PMLG

Polymer	BLG in polymer, mol %	Intensity ratio
PBLG	100	0.79
PBMLG	71	0.76
PBMLG	52	0.77
PBMLG	29	0.77
PMLG	0	0.79

Figure 4. Unit cell dimensions for PBLG (triclinic) and PMLG (hexagonal).

has been used¹² as a measure of helix content. In Table II are shown the intensity ratios obtained for the present copolymers PBMLG's, and homopolymers PBLG and PMLG.

Packing of PBMLG Helices in Unit Cells

As mentioned above, from the infrared spectra we have pointed out that molecules of the random copolypeptides PBMLG's exist in the α -helical conformation. In this section, the chain packing of these copolymer helices will be discussed as a function of copolymer composition by interpretation of X-ray diffraction patterns.

According to Bamford and Hanby¹³, the unit cell of PMLG was considered to be hexagonal with $a = b = 11.95 \text{ \AA}$ and c (fiber axis) $= 27.0 \text{ \AA}$. The most reliable information¹⁴ about the unit cell of PBLG may be that reported by Bamford, Hanby, and Happey¹⁵. These authors have suggested a triclinic cell, containing one chain, having the cell dimensions of $a = b = 15.25 \text{ \AA}$, c (fiber axis) $= 27.0 \text{ \AA}$, $\alpha = \beta = 84^\circ 20'$, and $\gamma = 122^\circ$, which may be regarded as a minor modification of the corresponding hexagonal cell. The cross section of the unit cell of PMLG and PBLG by plane perpendicular to the c -axis is shown in

Figure 5. X-ray diffraction photographs of PBLG, PBMLG's, and PMLG; $\text{CuK}\alpha$ radiation, flat photographic film. The beam was normal to the surface of the specimen, which was in a vertical plane. The direction of stretching was vertical. (a) PBLG [sample A8], (b) PBMLG (BLG, 81 mol%) [sample A7], (c) PBMLG (BLG, 47 mol%) [sample A4], (d) PBMLG (BLG, 19 mol%) [sample A2], and (e) PMLG [sample A1].

Table III. Distance between adjacent helices, $a(\text{\AA})$, calculated from X-ray data by assuming the hexagonal or triclinic cell, and the crystallographic density ρ

Polymer	BLG, mol%	$d_{\text{obs}}, \text{\AA}$	$a_{\text{calc}}, \text{\AA}$		ρ
			Triclinic	Hexagonal	
PBLG	100.0	13.26	15.16	15.31	1.28
PBMLG	81.0	12.92	14.76	14.92	1.25
PBMLG	47.1	12.06	13.79	13.93	1.24
PBMLG	29.0	11.43	13.07	13.20	1.26
PBMLG	19.0	10.87	12.13	12.55	1.29
PBMLG	0.0	10.34	11.82	11.94	1.30

Figure 4, in which the inner and outer circles schematically represent the effective cross sections of backbone and the whole chain helix, respectively.

The distance between the centers of adjacent helices was assumed to be equal to the unit cell dimension in the a -axis, and determined from (100) spacing for hexagonal and from (110) spacing for the triclinic cell. The X-ray diffraction photographs of copolymers PBMLG's and homopolymers PBLG and PMLG were taken with the beam perpendicular to the sample films cast from chloroform solution and oriented by stroking. The PBLG sample was found to indicate double orientation. Some of the photographs are shown in Figure 5. Degrees of orientation of all these films seem to be insufficiently high. However, the observed spacings can lead us to obtain the distance between adjacent helices. Calculations were carried out by assuming both the triclinic and hexagonal cells. The observed spacing and the calculated distance between adjacent helices are summarized in Table III. It should be emphasized that the numerical values obtained from both models are in accord within about 1% deviation. It is pointed out that these values are linearly increased with increasing BLG content of the polymer. In Table III, also, are included the crystallographic densities, ρ calculated from the volume, V (in $\text{\AA}^3$) of the unit cell and the number, n of molecules of molecular weight M in the unit cell by using the equation $\rho = 1.65 M_n/V$. The density values of the copolymers are slightly lower than those of homopolymers but the differences are not great. This means that the degrees of packing for copolymers are

slightly looser than those of homopolymers.

The identity period along the chain is the same for PBLG and PMLG. Further, both homopolymers do not significantly differ in their crystal systems. It is also known that fibrous and globular proteins possess crystal structures which allow different repeating units of the same general type to enter into the lattice. From the result given in Table III, it may be concluded that cocrystallization of the two monomer units has occurred. In other words, isomorphism is said to occur for the copolypeptides studied here over the complete composition range. Additional discussion on isomorphic replacement will be given in our succeeding paper from a stand point of the copolymer composition dependence on the helix-coil transition temperature.

Acknowledgement. This work was partly supported by a grant-in-aid from the Ministry of Education.

REFERENCES

1. C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides", Academic Press, New York, N. Y., 1956, Chapter II.
2. M. Szwarc, *Adv. Polym. Sci.*, **4**, 1 (1965).
3. E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, **78**, 941 (1956).
4. F. R. Mayo and F. M. Lewis, *J. Amer. Chem. Soc.*, **66**, 1594 (1944).
5. M. Fineman and S. D. Ross, *J. Polym. Sci.*, **5**, 269 (1950).
6. R. E. Nylund and W. G. Miller, *J. Amer. Chem. Soc.*, **87**, 3537 (1965).
7. T. Miyazawa, *J. Chem. Phys.*, **32**, 1647 (1960).
8. T. Miyazawa and E. R. Blout, *J. Amer. Chem.*

Synthesis and Structure Studies of Copolypeptides

- Soc.*, **83**, 712 (1961).
9. T. Miyazawa, Y. Masuda, and K. Fukushima, *J. Polym. Sci.*, **62**, S62 (1962).
10. Y. Masuda, *Kobunshi Kagaku (Chem. High Polymers)*, **20**, 161, 166, 171, 206, 210 (1963).
11. K. Itoh, T. Shimanouchi, and M. Oya, *Biopolymers*, **7**, 649 (1969).
12. Y. Masuda, T. Miyazawa, and M. Goodman, *Biopolymers*, **8**, 515 (1969).
13. C. H. Bamford and W. E. Hanby, *Nature*, **169**, 120 (1952).
14. A. Elliott, "Poly(α -amino acids)," G. D. Fasman, Ed. Marcel Dekker, New York, N. Y., 1967, p. 1.
15. C. H. Bamford, W. E. Hanby, and F. Happey, *Proc. Roy. Soc.*, **A205**, 30 (1951).