Conformation Studies of Copolypeptides Composed of γ -Benzyl-L-Glutamate and γ -Methyl-L-Glutamate in Solution

Toshio HAYASHI* and Akio NAKAJIMA**

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

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ABSTRACT: Chain conformation in solution was investigated for random copolypeptides composed of γ -benzyl-L-glutamate and γ -methyl-L-glutamate. From experimental results on the optical rotatory dispersion associated with thermally induced coil-to-helix transition in dichloroacetic acid—dichloroethane systems, it has been concluded that these copolypeptides exist in perfectly helical form in analogy with homopolypeptides. Thermodynamic parameters for the transition, and the effect of copolymer composition on the transition temperature, were also discussed.

KEY WORDS Polypeptide / Poly(γ -Benzyl-L-Glutamate—Co- γ -Methyl-L-Glutamate) / Chain Conformation in Solution / Optical Rotatory Dispersion / Coil-to-Helix Transition / Cooperative Parameter /

In our previous papers,¹⁻³ we investigated the molecular conformations of homopolypeptides, poly(γ -methyl-L-glutamate) (PMLG) and poly(γ -benzyl-L-glutamate) (PBLG), in various solvents, such as dichloroacetic acid (DCA), trifluoroacetic acid (TFA), *m*-cresol and some mixed solvents, and discussed the influence of temperature on molecular conformation in these systems.

It is known that the conformation of some synthetic polypeptides in solution is transformed from coil to helix with increasing temperature through an intermediate stage called interrupted helix.⁴⁻¹⁰ Such inverse transition has been found, for example, in the systems: PBLG in mixtures of DCA and 1,2-dichloroethane (DCE);^{4-6,8} in mixtures of DCA and 1,4-butylene dichloride;⁹ in mixtures of DCA and octyl chloride,⁹ and in mixtures of DCA and 1-dodecyl chloride;⁹ PMLG in mixtures of DCA and DCE,³ and poly(ϵ -carbobenzoxy-L-lysine) (PCBL) in mixtures of DCA and chloroform,⁹ and in mixtures of DCA and DCE.¹¹

PBLG, PMLG, and PCBL have some common characteristics due to their large side

chain groups. Comparing these three polymers in respect of DCA content in DCA—DCE mixtures, which give the same transition temperature, we pointed out³ that PBLG undergoes transition at the highest content of DCA, the hydrogen-bond rupturing component. The helix stability of PBLG is highest among these three polymers, and it was also pointed out that the number of residues cooperatively contributing to transformation is larger for PBLG than for PMLG.

The present paper deals with experimental results on the variations of optical rotatory dispersion associated with thermally induced coil-to-helix transition of the random copolymers PBMLG, composed of γ -benzyl-L-glutamate and γ -methyl-L-glutamate, in DCA—DCE mixtures. The results obtained are compared with those for PMLG and PBLG homopolymers. The enthalpy of transition ΔH of these homopolymers and copolymers in DCA—DCE systems is also estimated and discussed with respect to the helix stability of these polymers.

EXPERIMENTAL

Materials

The samples of copolypeptides (PBMLG) used

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

** To whom correspondence should be addressed.

Table I. Polymer composition and degrees of polymerization^a

Designation	Polymer composition		$[\eta]$, dl/g	P_n
	BLG	MLG		
P 00	0.0	100.0	2.78	2200
P 05	4.5	95.5	2.41	1805
P 10	8.7	91.3	2.10	1490
P 20	19.0	81.0	2.00	1390
P 30	29.0	71.0	1.72	1130
P 40	41.6	58.4	1.54	970
P 50	52.1	47.9	1.33	790
P 60	59.8	40.2	1.22	700
P 701	71.2	28.8	0.83	410
P7011	71.4	28.6	2.02	1410
P 801	81.1	18.9	0.60	260
P8011	81.0	19.0	2.21	1600
P 901	88.3	11.7	0.58	250
P9011	88.2	11.8	2.30	1690
P 100	100.0	0.0	1.25	730

^a P_n of the samples used. P_n was estimated from $[\eta]$ by using the equation²⁵ given for poly(γ -benzyl-L-glutamate).

in the present study were synthesized by us.¹² The chemical compositions and the limiting viscosity numbers in DCA (25°C) of these copolymers are listed in Table I.

The solvents used were dichloroacetic acid (DCA) and 1,2-dichloroethane (DCE). These solvents were purified by the usual means described in the literature and finally distilled by the use of a long column.

Measurements

The limiting viscosity number $[\eta]$ (dl/g) of the copolymers was determined in DCA at 25°C by using Ubbelohde type capillary viscometers.

Optical rotatory dispersions in a temperature range from 5 to 60°C were measured with a Yanagimoto OR-100 type Spectro-Polarimeter using a tungsten lamp as the light source. The wave lengths used ranged from 325 to 610 m μ . The concentration of copolymers solutions was 1.0 g/dl throughout these measurements. The solvent systems used were DCA—DCE (50- to 100-mol% DCA) mixtures.

RESULTS AND DISCUSSION

The optical rotatory dispersions of these co-

polypeptides were measured in DCA—DCE mixtures. The results are examined in terms of the helix content X^H calculated in two different ways. One method is based on¹³ the coefficient b_0 in the Moffitt equation¹⁴

$$[\alpha] = \left(\frac{100}{M_0} \right) \left(\frac{n^2 + 2}{3} \right) \left[\frac{a_0 \lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0 \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \right] \quad (1)$$

where a_0 is a constant which may be expected to vary with the nature of the side chain of polypeptide and to depend on the kind of solvent, whereas the parameter b_0 is a function of the helix content. Further, M_0 is the molecular weight per peptide residue, n the refractive index of the solvent and $\lambda(m\mu)$ the wave length of the light source. In our previous analysis of the dispersion data^{1,2} on PMLG and PBLG in some solvents in which the helical conformation is stable, we obtained $b_0 = -600$ and $\lambda_0 = 212 m\mu$. The helix content X^H , in this case, is estimated from

$$X^H = \frac{b_0 - b_{0,c}}{b_{0,h} - b_{0,c}} \quad (2)$$

where, $b_{0,h}(= -600^\circ)$ and $b_{0,c}(= 0^\circ)$ denote the b_0 -values for perfect helix and coil, respectively.

The other method is based on the coefficients in the modified two-term Drude equation,¹⁵

$$[m'] = \frac{A_{193} \lambda_{193}^2}{\lambda^2 - \lambda_{193}^2} + \frac{A_{225} \lambda_{225}^2}{\lambda^2 - \lambda_{225}^2} \quad (3)$$

which gives the helix content X^H by the following equation

$$X^H = \frac{(A_{193} - A_{225}) + I}{K} \quad (4)$$

where A_{193} and A_{225} are the A -values at 193 and 225 m μ , respectively, $I=650$, and $K=5580$.

The values of X^H so obtained from eq. 2 were in close agreement with those from eq. 4. Consequently, in this paper, we shall use the b_0 value as a measure of helix content.

Effect of Solvent Composition on Transition

In order to test the stability of the copolypeptides compared with that of the homopolymers, the optical rotatory dispersions of three copolypeptides containing 19-, 52-, and 81- mol% γ -benzyl-L-glutamate (BLG) were examined in DCA—DCE mixtures as functions of solvent composition.

Figure 1. Effect of solvent composition on coil-to-helix transition at 25°C in DCA—DCE mixtures, for (1) PBLG [Sample P100] (2) PBMLG (81-mol% BLG) [Sample P801], (3) PBMLG (52-mol% BLG) [Sample P50], (4) PBMLG (19-mol% BLG) [Sample P20], and (5) PMLG [Sample P00].

Figure 1 shows the relation between the solvent composition and b_0 for these copolypeptides and for homopolypeptides, PMLG and PBLG. It should be emphasized that even in the case of copolypeptides (PBMLG) the b_0 value at lower DCA contents are almost the same as that ($b_0 = -600$) of homopolypeptides (PBLG and PMLG) and it decreases to $b_0 = 0$ with increasing DCA content. Figure 1 also illustrates the fact that different amounts of DCA are required to destroy helical conformation at a given temperature. In the molecular weight range used here, the difference in molecular weight may not significantly affect the transition point.

In our earlier studies,^{2,3} we concluded that PMLG undergoes a coil-to-helix transition at a lower concentration of DCA in DCA—DCE mixtures than does PBLG under the conditions which give the same transition temperature, namely, the helix of PBLG is considered to be more stable than that of BMLG. It is of interest that helices of these copolypeptides are more stable than those of PMLG, but are less stable than those of PBLG. Thus the side chain may play an important role in helix stabilization.

Effect of Temperature on Transitions

We will now turn to the conformational be-

Figure 2. Temperature dependence of b_0 in a 30-vol% DCE—70-vol% DCA mixture, for (1) PBLG [Sample P100], (2) PBMLG (88-mol% BLG) [Sample P901], (3) PBMLG (52-mol% BLG) [Sample P50], (4) PBMLG (19-mol% BLG) [Sample P20], and (5) PMLG [Sample P00].

havior of these copolypeptides resulting from changes in temperature. Figure 2 shows the temperature dependence of b_0 of these copolypeptides. The solvent system used was a 30-vol% DCE—70-vol% DCA mixture. It is obvious from Figure 2 that the transition temperature shifts continuously toward higher temperatures as the content of MLG-residues in the copolypeptide becomes higher. However, the sharpness of the transition curve of these copolypeptides is almost the same as those of PMLG and PBLG homopolymers. Further, all of these transition curves asymptotically approach *ca.* -600° for b_0 with increasing temperature. We can, therefore, conclude that these copolypeptides, in which the two different amino acid residues are distributed randomly,¹² are able to exist in a perfectly helical form in this solvent system.

Comparison of Transition Behavior of Copolypeptide PBMLG with that of Blend of Homopolymers PBLG and PMLG

Figure 3 shows the transition curves of the copolypeptide (52-mol% BLG—48-mol% MLG) and of a 50—50 blend of PBLG and PMLG. The solvent system used was a 30-vol% DCE—70-vol% DCA mixture. Inverse transitions were

Figure 3. Temperature dependence of b_0 in a 30-vol% DCE–70-vol% DCA mixture, for copoly-peptide (curve b) containing 52-mol% BLG, and for a polymer blend (curve a) containing 50-mol% PBLG and 50-mol% PMLG.

observed in both cases. In the case of the blend, two transition temperatures were observed, while in the case of the copoly-peptide, only one transition temperature was observed at about the middle point of the two transition temperatures observed for the former.

The lower transition temperature of the blend polymer about at 8°C corresponds to that of PBLG, and the higher one at about 30°C corresponds to that of PMLG. Comparison of these two transition curves may suggest that, in the case of the blend, the transition takes place independently for PBLG and PMLG, while, in the case of this copolymer, the transition takes place cooperatively. The copolymer used here is a random copolymer as mentioned in the preceding section. However it is difficult, at this stage, to attribute the observed behavior to the random arrangement of the comonomers only.

Thermodynamic Parameters for Transition

Experimental data relating to the thermally induced conformational transition have been analyzed in terms of the Zimm–Bragg^{16–18} theory, in which two distinct transition enthalpies are of significance. These are: ΔH , the van't Hoff heat of transition, derived from the overall change in helix content of the poly-

peptide as a function of temperature; and ΔH_{res} , the enthalpy change accompanying the formation of one mole of intramolecular hydrogen bonds, *i.e.*, the enthalpy change on converting one mole of amino acid residues from the random coil to the helical form. In the formulation of Zimm and Bragg¹⁷ and of Applequist,¹⁸ ΔH is related to ΔH_{res} by the following equation

$$\Delta H = \frac{\Delta H_{\text{res}}}{\sigma^{1/2}} \quad (5)$$

The parameter σ is designated as the cooperative parameter by Achermann and Neumann.²⁰

The helix content X^H was obtained as a function of temperature at a fixed solvent composition, hence the value of ΔH is readily determined experimentally from the slope of the b_0 vs. temperature curve using the equation^{3,18,19}

$$\left(\frac{dX^H}{dT} \right)_{T_t} = \frac{1}{(b_{0,h} - b_{0,c})} \left(\frac{db_0}{dT} \right)_{T_t} = \frac{\Delta H}{4RT_t^2} \quad (6)$$

On the other hand, the value of ΔH_{res} can be directly measured by the calorimetric technique,^{8,20–22} and, alternatively, can be determined by the method proposed by Karasz, *et al.*⁹ According to Karasz, DCA molecules bound to the polypeptide have lost their translational freedom at temperatures below T_t , and are therefore in a “solid” phase. Above T_t , however, these DCA molecules are released and are in normal liquid state. At T_t , the chemical potential μ_{DCA}^s of the DCA in the “solid” state equals that of μ_{DCA}^l of DCA in the liquid state in the DCA–DCE mixture. From the ideal solution approximation the following expressions are derived:

$$\begin{aligned} \mu_{\text{DCA}}^l - \mu_{\text{DCA}}^0 &= -RT \ln X_{\text{DCA}} \\ \mu_{\text{DCA}}^s - \mu_{\text{DCA}}^0 &= -\Delta H_{\text{DCA}} \left(1 - \frac{T_t}{T_t^0} \right) \end{aligned}$$

from which we obtain the equation

$$\frac{d(\ln X_{\text{DCA}})}{dT_t} = \frac{\Delta H_{\text{DCA}}}{RT_t^2} \quad (7)$$

where μ_{DCA}^0 is the chemical potential of the pure DCA; X_{DCA} is the mole fraction of DCA in the solvent mixture; T_t^0 is the transition temperature of the polypeptide in DCA in the absence of DCE, and ΔH_{DCA} is the overall heat,

Table II. Thermodynamic parameters of copolypeptide PBMLG (52-mol% BLG) and homopolypeptides PBLG and PMLG in DCA—DCE mixtures

Polymer	P_n	X_{DCA}	T_i , °C	ΔH , kcal/mol	ΔH_{res} , cal/mol	ΔS_{res} , e.u.	$\sigma \times 10^4$
PBLG	730	0.69	6	90 ± 10	930 ± 100	3.4 ± 0.3	1.0
PBMLG (BLG 52%)	790	0.69	17	65 ± 10	910 ± 100	3.1 ± 0.3	2.0
PMLG	2200	0.69	28	85 ± 10	910 ± 100	3.0 ± 0.3	1.2

per mole of DCA, associated with the transition of "solid" DCA.

Eq. 7 was derived for the transition of DCA from "solid" state to "liquid" state. The total number of hydrogen bonds is not changed throughout the transition, and furthermore there is one-to-one molar equivalence between the bonded DCA molecules and the peptide residues on the average, and the "melting point" of bonded DCA ("solid") is equated to the transition temperature T_i of the polypeptide. Hence ΔH_{DCA} can be identified with the enthalpy of transition ΔH_{res} . Accordingly,

$$\frac{d(\ln X_{\text{DCA}})}{dT_i} = \frac{\Delta H_{\text{res}}}{RT_i^2} \quad (8)$$

Now we estimate ΔH and ΔH_{res} by the use of eq 6 and 8, respectively, for PMLG, PBMLG (52-mol% BLG), and PBLG in DCA—DCE mixtures. ΔH_{res} was estimated from Figure 4, which showed the relation between the transition temperature T_i and the logarithm of the mole fraction of DCA in the mixture. Final-

Figure 4. Transition temperature as a function of solvent composition X_{DCA} (=mol fraction of DCA), for (1) PBLG [Sample P100], (2) PBMLG (52-mol% BLG) [Sample P50], and (3) PMLG [Sample P00].

ly the values of σ were calculated from eq 5. Table II is a summary of these data. The relation $\Delta S_{\text{res}} = \Delta H_{\text{res}}/T$ was used to estimate the conformational entropy change ΔS_{res} per peptide residue.

It has not been revealed whether σ is independent of solvent or not. Comparing at the same solvent composition, it seems from the results shown in Table II that the larger values ($\sigma = 2 \times 10^{-4}$) of σ for the present copolypeptides than those for PBLG ($\sigma = 1.0 \times 10^{-4}$) and PMLG ($\sigma = 1.2 \times 10^{-4}$) suggest that the transition of the present copolypeptides is performed less cooperatively than that of the corresponding homopolymers. These σ values, however, may be regarded as practically constant within the limits of experimental error.

The values of the enthalpy change ΔH_{res} associated with the formation of an intramolecular hydrogen bond in the copolypeptides were also about the same as those for the homopolymers. It may be concluded that the enthalpy contribution of the intramolecular hydrogen bond formation, in this case, is not affected by the difference in atomic groups, methyl and benzyl, which are located far from the backbone chain.

The effect of the degree of polymerization on the estimated values of σ and ΔH_{res} was not examined. Recently, Teramoto, *et al.*,^{23,24} proposed a method of obtaining σ and ΔH_{res} from the helix content at fixed solvent and temperature conditions as a function of the degree of polymerization, on the basis of Nagai's theory for the helix—coil transition.

Effect of the Degree of Polymerization on T_i

In our previous paper,³ we discussed the effect of the degree of polymerization on the transition temperature T_i of both PMLG and PBLG. It was found that the transition temperature shifted toward lower temperatures and, at the same time, the transition became sharper as

Figure 5. Reciprocal of transition temperature in a 30-vol% DCE—70-vol% DCA plotted against reciprocal of degrees of polymerization, for PBLG, PBMLGs, and PMLG. Numerals in this figure denote the BLG mol%.

Figure 6. Relation between the transition temperature T_t , for $P_n = \infty$ and copolymer composition given by BLG mol% for copolypeptides PBMLGs.

the degree of polymerization of the polypeptide increased. The degree of polymerization of copolypeptides used in this paper is different in each case. Therefore, the b_0 value at constant solvent composition was measured as a function of temperature for copolypeptides and homopolypeptides having different degree of polymerization, to obtain the transition temperature T_t as a function of the degree of polymerization P_n . Figure 5 shows plots of $1/T_t$ in DCA—DCE mixtures *vs.* $1/P_n$ for these co-

polypeptides and the homopolypeptides PBLG and PMLG.

The plots of $1/T_t$ *vs.* $1/P_n$ for PBLG and PMLG are represented by straight lines with almost identical slopes. The slopes for the PBMLG copolypeptides, whose BLG contents are 88, 81, and 71 mol%, respectively, are also the same as those for PBLG and PMLG. For copolypeptides with BLG contents from 60 to 10 mol%, the experimental point is only one for each. In this case we may assume straight lines with the same slope as the homopolymers for these copolymer series.

The straight lines so drawn were extrapolated to $1/P_n \rightarrow 0$, giving the transition temperature $T_{t,\infty}$ for polymers with infinite chain length $P_n = \infty$, which is characteristic of the special copolypeptide considered, and may be compared with the melting points of crystalline copolymers as functions of copolymer composition. Figure 6 shows the plot of $T_{t,\infty}$ *vs.* the BLG content (mol%) in PBMLG copolypeptides. The plot is approximately represented by a straight line.

It is known that some proteins possess crystal structures that allow different amino acid residues to enter into the same crystalline lattice. Such phenomenon designated as cocrystallization has also been observed among vinyl copolymers²⁶ and copolyamides,²⁷⁻²⁹ even when the corresponding homopolymers possess different, but not markedly different, crystalline structures.

The random introduction of isomorphous units results in a linear variation of the melting point between values of the pure individual homopolymers. Such behavior in melting point curves is quite different from that for random copolymers in which only one of the comonomers crystallizes, and for block copolymers.²⁶

The linear relation between the transition temperature T_t and the mole fraction of BLG in the copolypeptide shown in Figure 6 is similar to the melting point curve for isomorphous copolymer systems. In our preceding paper,¹² we pointed out that from X-ray diffraction studies on solid samples it was learned that each copolypeptide PBMLG cocrystallizes in a definite crystalline form regardless of its BLG content, though the crystalline modification of

PBLG (triclinic) was somewhat different from that of PMLG (hexagonal). That the molecules of these copolypeptide in solution can exist in perfectly helical form has also been confirmed by the optical rotatory experiments mentioned above. The experimental result given in Figure 6 is consistent with the structural and conformational information discussed above concerning the present copolypeptides.

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