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Synthesis and Conformation Studies of Copolypeptides Composed of γ-Benzyl-L-Glutamate and ε-*N*-Carbobenzoxy-L-Lysine

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Copolypeptides composed of γ-benzyl-L-glutamate and ε-*N*-carbobenzoxy-L-lysine covering the whole range of copolymer compositions were synthesized by the NCA method. The experimentally obtained monomer reactivity ratios, r_1 (for BLG)=1.8 and r_2 (for CBL)=0.5, 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. Chain conformation in solution was investigated for these copolypeptides. From experimental results on the optical rotatory dispersion regarding thermally induced coil-to-helix transition in dichloroacetic acid-1,2-dichloroethane systems, it was concluded that these copolypeptides exist in helical form in solution in analogy with homopolypeptides. Thermodynamic parameters for the transition, and the effect of copolymer composition on the transition temperature, were also discussed.

INTRODUCTION

In our previous papers,[1,2] we investigated the molecular conformation of copolypeptides composed of monomers having similar side chains, *i.e.*, copolypeptides composed of γ-benzyl-L-glutamate(BLG) and γ-methyl-L-glutamate(MLG), for samples covering whole composition range. The experimentally obtained monomer reactivity ratios, r_1(for BLG)=0.70 and r_2(for MLG)=1.40, suggested that the copolymers formed were regarded approximately as random copolymer. Further, from experimental results on the optical rotatory dispersion(ORD) in thermally induced coil-to-helix transition in dichloroacetic acid(DCA)-1,2-dichloroethane (DCE) systems, it has been concluded that these copolymer can exist in perfectly helical form in DCE-rich DCA-DCE mixture just as homopolypeptides. Since all the thermal transitions for copolymers of BLG and MLG appeared in rather narrow temperature range, an unique solvent mixture was used throughout without being worried by possible influence due to difference in solvent composition.

In the present work, we will concern with copolypeptides composed of γ-benzyl-L-glutamate(BLG) and ε-*N*-carbobenzoxy-L-lysine(CBL). In this case, it is known that the transition temperature of homopolypeptide PBLG is far apart from that of PCBL, therefore, it is necessary to use different solvent mixtures in order to bring the transitions into available temperature range.

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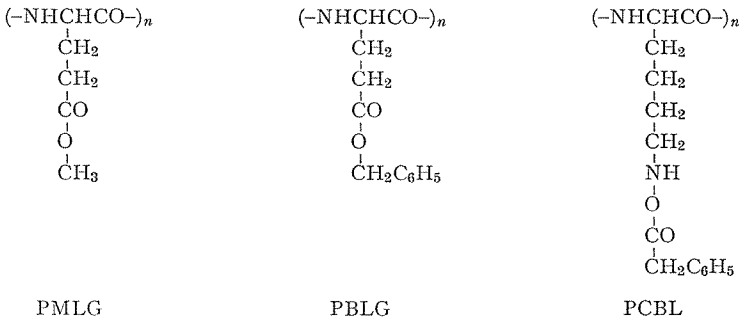


PMLG PBLG PCBL

Copolypeptides(PBGCL) of γ-benzyl-L-glutamate(BLG) and ε-*N*-carbobenzoxy-L-lysine(CBL) covering comonomer ratio from 0 to 1 were synthesized by the NCA method initiated by triethylamine(TEA). TEA is known to yield polypeptides with high degrees of polymerization. The copolypeptide composed of γ-benzyl-L-glutamate and ε-*N*-carbobenzoxy-L-lysine has already been synthesized by Y. Shalitin and E. Katchalski[3] with diethylamine initiator in dimethylformamide(DMF), and by A. Roig *et al.*[4] with sodium methoxide in dioxane. Results obtained on our systems will be compared with those of these authors. Further, thermal transition of these copolypeptides will be discussed in comparison with that of other copolypeptide systems.

EXPERIMENTAL

Synthesis of Copolypeptides

The monomers, *N*-carboxy-γ-benzyl-L-glutamate(BLG-NCA) and *N*-carboxy-ε-*N*-carbobenzoxy-L-lysine(CBL-NCA) were prepared by the method proposed by Blout and Karlson,[5] and purified by repeated recrystallization from an ethyl acetate solution with the addition of petroleum ether. The BLG-NCA and CBL-NCA, in desired mole ratios, were dissolved in a 1 : 1(v/v) mixture of dry dioxane and ethylendichloride. The total anhydride concentration was kept at 2%. The polymerization was initiated with triethylamine(TEA) at an anhydride: initiator ratio([M]/[I]) of 100. The polymerization was completed within about 48 hrs at 25°C.

Samples in series C were allowed to polymerize until no further evolution of CO_2 gas was observed, *i.e.,* up to a high conversion level. Samples in series D were stopped polymerizing at a lower conversion stage so as to obtain the monomer reactivity ratios. The copolypeptides formed were precipitated in a large amount of cold methanol and dried under reduced pressure at 50°C.

Composition of these copolypeptides was determined from the analytical values of N-atom. These elemental analysed were carried out in the Organic Microanalysis Center in Kyoto University.

The limiting viscosity number [η] as a measure of molecular size of these copolypeptides was determined in 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 sum-

Table 1. Copolymerization of γ-Benzyl-L-Glutamate with ε-*N*-Carbobenzoxy-L-Lysine by the NCA Method

Sample	[M]/[I]*	Initial Monomer Ratio, mol%-BLG	Polymer Composition mol%-BLG	[η](dl/g) (DCA, 25°C)	Conv. %
C-1	100	100.0	100.0	1.90	72.1
2	100	79.5	81.1	1.57	69.6
3	100	52.1	53.5	1.75	71.5
4	100	44.0	45.7	1.93	63.5
5	100	25.5	27.0	1.88	52.4
6	100	0.0	0.0	2.20	81.2
D-1	50	79.4	83.2	0.50	32.4
2	50	79.4	82.3	—	52.4
3	50	60.0	68.3	0.57	27.4
4	50	46.0	53.9	0.69	32.4
5	50	21·0	27.0	0.82	26.3

* Monomer-initiator mole ratio

marized in Table 1, in which series C and D, 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 a spectral region of 400–4000 cm^{-1}.

Optical Rotatory Dispersions

Optical rotatory dispersions in a temperature range from 5 to 50°C were measured with a Yanagimoto OR-100 type spectropolarimeter using a tungsten lamp as the light source. The wave lengths used ranged from 325 to 610 mμ. The concentration of copolymer solutions was 1.0 g/dl throughout these measurements. The solvent system used was DCA-DCE mixture.

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 ε-*N*-carbobenzoxy-L-lysine(CBL) at conversion level about 30%, being taken from the data on series D in Table 1. As is obvious from Figure 1, BLG was slightly more reactive than CBL but the deviation from the linear relationship was not so large.

Now we denote the monomer reactivity ratios by r_1 and r_2 for the copolymerization of BLG with CBL. To obtain the monomer reactivity ratios, the method proposed by Fineman and Ross[6] was employed. The numerical values of r_1 and r_2 obtained from the

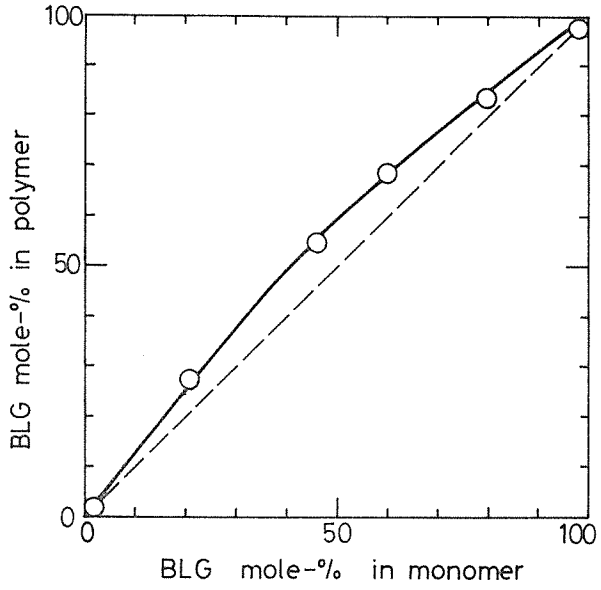


Fig. 1. Copolymer composition curve for PBGCL copolymer at about 30% conversion. Data are taken from series D in Table 1.

data shown in Table 1 were as follows,

$$r_1(\mathrm{BLG}) = 1.8$$
$$r_2(\mathrm{CBL}) = 0.5$$

Shalitin *et al.*[3] investigated the diethylamine-initiated copolymerization of BLG with CBL in DMF. They found that the conversion rate was practically equal to the sum of the conversion rate of the individual monomer. The data reported by them[3] demonstrate that the mole ratio of glutamate acid to lysine residues in all the copolymers investigated was approximately 2 to 2.5 times greater than the mole ratio of BLG to CBL in the initial monomer mixture. While, in our data, the ratio of glutamic acid to lysine residues in all the copolymers investigated is about 1.5 to 2.0 times greater than the mole ratio in the initial monomer mixture. The difference between our data and Shalitin's may be attributed to the nature of initiator and solvent for polymerization, though the difference is not so large.

Since $r_1(\mathrm{BLG}) > r_2(\mathrm{CBL})$, it can be presumed that the polymer chains formed at the beginning of the reaction are rich in the more reactive component, glutamate residue. Since the more reactive monomer, the glutamate NCA, is consumed more rapidly than the lysine NCA, polymer chains formed in the last reaction stage are composed mainly of the less reactive species, lysine. Thus the copolypeptide is not perfectly random; the difference in rate constants produces a "concentration gradient" along the copolypeptide conversion. In Fig. 2 is shown the BLG mol% in copolymer against the per cent polymerization for the systems derived from 79.4 : 20.6 mol% monomer mixture. It is also clear that the amount of the BLG residues in the copolymer formed decrease with increasing conversion. As a result, distribution of each monomer residue in the resulting copolypeptide chains is not completely random.

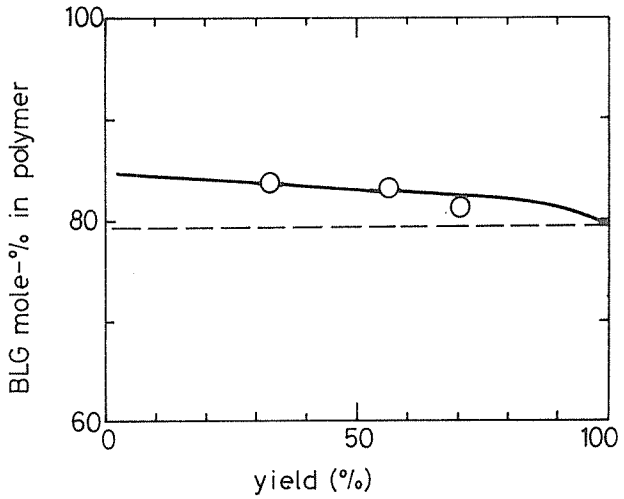


Fig. 2. Copolymer composition *vs.* conversion curve for 79.4 mol%-BLG–20.6 mol%-CBL system. The points represent the data of D-1, D-2, and C-2 in Table 1, respectively, from left to right.

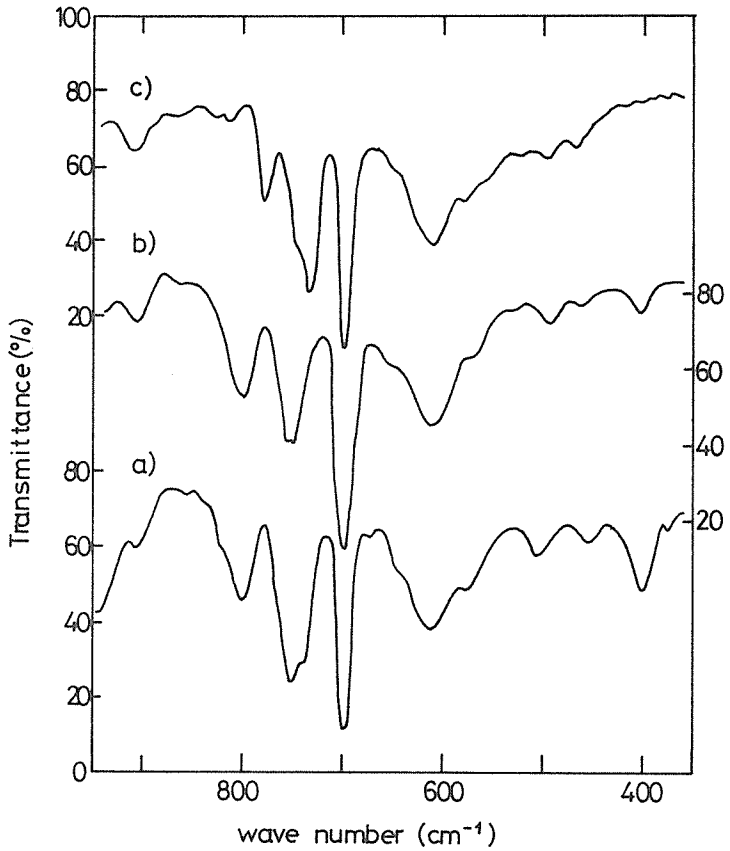


Fig. 3. Infrared spectra for (a) PBLG (Sample C-1), (b) PBGCL (Sample C-4, 45.7 mol%-BLG), and (c) PCBL (Sample C-6).

Chain Conformation of Copolypeptide PBGCL in Solid State

Infrared spectra were measured with solid film of copolypeptides PBGCL's with different copolymer compositions. Some typical illustrations of these spectra in the region of 400–900 cm^{-1} were shown in Fig. 3 together with those of homopolymers PBLG and PCBL.

Of the low-frequency modes, the amide V vibration has been most useful in structural investigations. It involves N-H out-of-plane bending and depends considerably on the backbone conformation. The α-helical and disordered forms may now be distinguished even for unoriented films, and the fraction of the α-helical form may be estimated in the presence of the disordered form.

According to Miyazawa *et al.*,[7,8] the amide V band of the α-helical form appeared at 610–620 cm^{-1} for PBLG, PMLG, and PCBL, while that of the disordered form at 650 cm^{-1}. As is obvious in Fig. 3, the amide V band for copolypeptides PBGCL's appeared at 615 cm^{-1}, just at the same wave number as that for PBLG and PCBL, with almost the same order of peak intensity as that of homopolypeptides. Such a result means that these PBGCL's exist in helical conformation and, moreover, the helix content of these copolypeptides is nearly the same as that of homopolypeptides.

Estimation of Helical Content in Solution

The optical rotatory dispersions(ORD) of these copolypeptides were measured in DCA–DCE mixtures. The results are examined in terms of parameters appeared in the Moffitt equation,[9]

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

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. 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.

While, the other method is based on the coefficients in the multi-term Drude equation.[10]

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

Since $A_{(193)}$ includes the contributions of two Cotton effects lying at two different Cotton effects at 193 $m\mu$ for α-helical form and 198 $m\mu$ for random conformation, eq. 2 is called a modified two-term Drude equation. The plot of $[m'](\lambda^2-\lambda_{193}^2)/\lambda_{193}^2$ against $\lambda_{225}^2/(\lambda^2-\lambda_{225}^2)$ should yield a straight line, of which $A_{225}(\lambda_{225}^2-\lambda_{193}^2)/\lambda_{193}^2$ is the slope and $A_{(193)}+A_{225}(\lambda_{225}^2/\lambda_{193}^2)$ is the intercept. Using the analysis given above, we have calculated $A_{(193)}$ and A_{225} for copolypeptides and component homopolypeptides. The modified two-term Drude equation yields two independent parameters, $A_{(193)}$ and A_{225}, and thus permits the detection of other ordered structures when the relation between the two parameters deviates from that expected for a mixture of α-helical and random conformation. The results of analysis were shown in Fig. 4, in which all points fall on an identical straight line. This fact shows that no structure is present other than α-helical and random coil conformation,

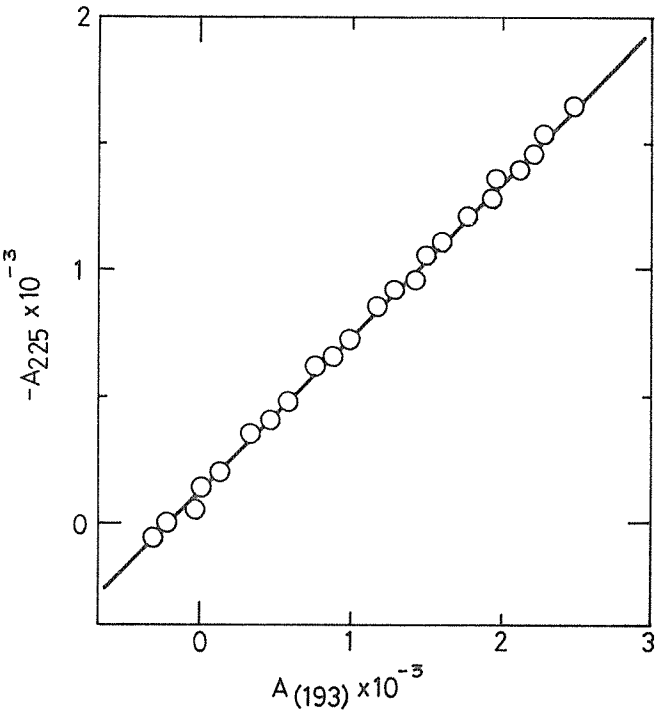


Fig. 4. Plot of $A_{(193)}$ versus A_{225} for copolypeptides in DCA-DCE mixtures. Values of A's were obtained from the thermal transitions of the copolypeptides included in Table 1. The polymer concentration is 1 g/dl.

and allows us to use the same numerical values given for both homopolypeptides for the calculations of the helical content X^H.

The helical content X^H, using λ_0=212 mμ in eq. 1, was estimated from

$$X^H = \frac{b_0 - b_{0,c}}{b_{0,h} - b_{0,c}} \tag{3}$$

where, $b_{o,c}$ and $b_{o,h}$ are characteristics for perfect coil and perfect helix, respectively. The other method is based on eq. 2, which gives the helix content X^H by the following equation.[10]

$$X^H = \frac{(A_{(193)} - A_{225}) + 650}{5580} \tag{4}$$

The values of X^H so obtained from eq. 3 were in agreement with those from eq. 4. Consequently, in this paper, we shall use the b_o values as a measure of helix content.

Comparison of Transition Behavior of Copolypeptide PBGCL with that of Blend of Homopolypeptides PBLG and PCBL

Figure 5 shows the transition curves of PBGCL C-4(45.7 mol%-BLG-54.3 mol%-CBL) and of a 50–50 blend of PBLG and PCBL as a function of solvent composition at 25°C. The solvent mixtures used were DCA-DCE systems. In the case of the blend, two transition regions were observed, while in the case of the copolypeptide, only one

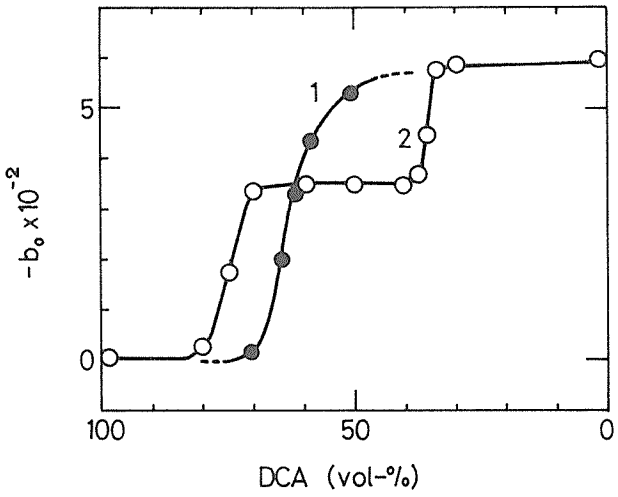


Fig. 5. Solvent composition dependence of b_0 at constant temperature, 25°C, for copolypeptide (curve 1) containing 45.7 mol%-BLG, and for a polymer blend (curve 2) containing 50 mol%-PBLG-50 mol%-PCBL.

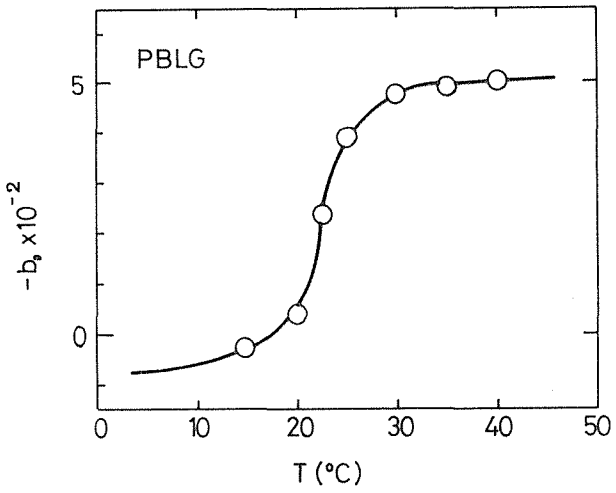
transition region was observed at about the middle point of the two transition regions observed for the blend. With respect to the polymer blend, the transition region at about 37 mol%-DCA corresponds to that of PCBL, and that at about 75 mol%-DCA corresponds to that of PBLG. Comparison of these transition curves for the copolymer and the polymer blend may suggest that, in the case of the blend, the transition takes place independently for PBLG, and PCBL while, in the case of the copolymer, the transition takes place cooperatively.

Effects of Solvent Composition and Temperature on Transition

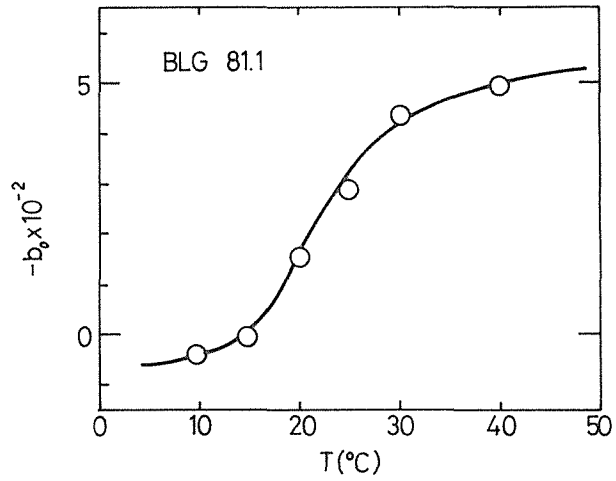
We will now turn to the conformational behavior of these copolypeptides resulting from change in temperature. Since the homopolypeptides, PBLG and PCBL, are rather different in helix stability, it was impossible to use an unique solvent mixture of fixed composition to compare the thermal transition curves of these copolypeptides. In order to keep the transitions within an available temperature range, different solvent compositions

Table 2. Composition of Solvent Mixture DCA-DCE and ΔH, the Van't Hoff Heat of Transition, for Thermal Transition.

Sample	Polymer Composition (mol% of BLG)	DCA (vol%)	ΔH (kcal/mol)
C-1	100.0	75.0	80±10
C-2	81.1	70.0	65±10
D-3	68.3	66.7	40± 5
C-4	45.7	61.5	25± 5
C-5	27.0	54.5	20± 5
C-6	0.0	37.7	75±10



(a) Temperature dependence of b_0 in a 75.0 vol%-DCA-25.0 vol%-DCE mixture for PBLG (Sample C-1).



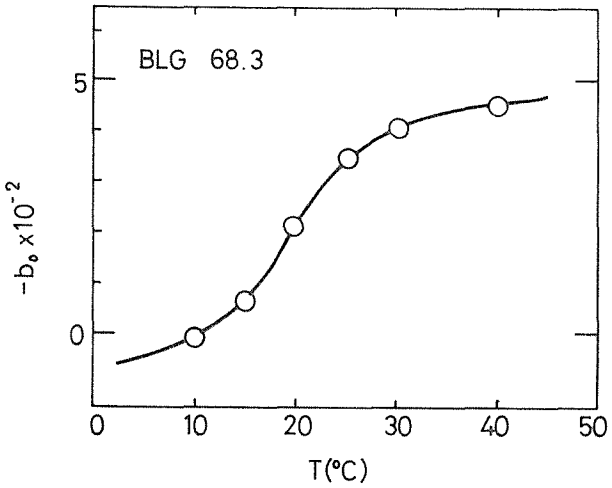
(b) Temperature dependence of b_0 in a 70.0 vol%-DCA-30.0 vol%-DCE mixture for PBGCL copolymer (Sample C-2, 81.1 mol%-BLG).

Fig. 6.

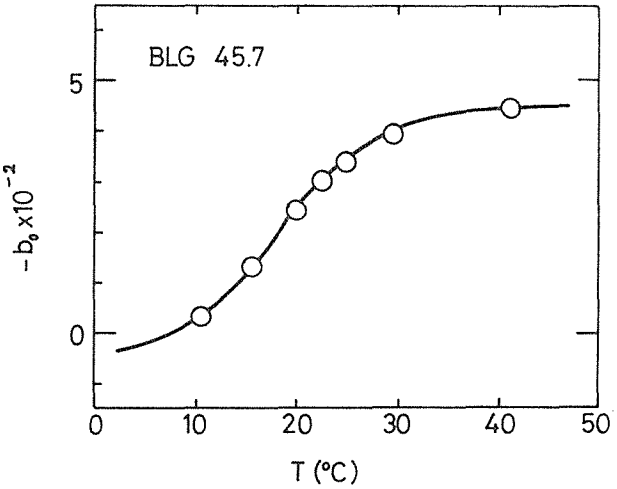
were used. The experimental results were shown in Table 2. In Figs. 6 a–6 f, experimentally observed thermal transition was illustrated for samples C-1, C-2, D-3, C-4, C-5, and C-6. The sharpness of transition was quite different from case to case.

It is clear that the amount of DCA needed to bring the transitions within the same temperature range for the copolypeptides are intermediate between those of the pure homopolypeptides, and so are the stabilities of the helices, although not necessarily in a linear way.

Experimental data relating to the thermally induced conformational transition have been analyzed in terms of the Zimm-Bragg theory,[11~13] in which two distinct transition



(c) Temperature dependence of b_0 in a 66.7 vol%-DCA-33.3 vol%-DCE mixture for PBGCL copolymer (Sample D-3, 68.3 mol%-BLG).

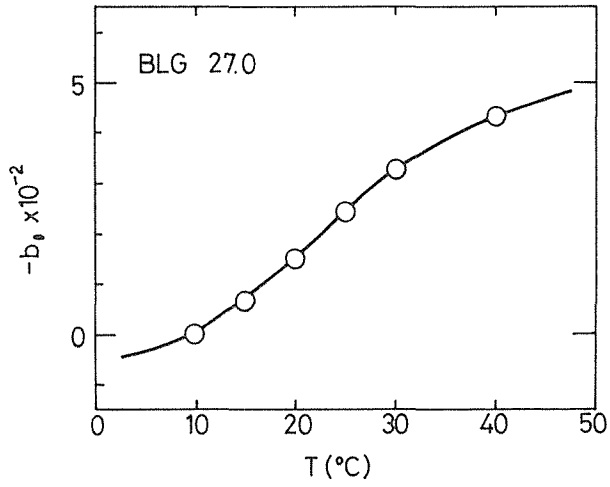


(d) Temperature dependence of b_0 in a 61.5 vol%-DCA-38.5 vol%-DCE mixture for PBGCL copolymer (Sample C-4, 45.7 mol%-BLG).

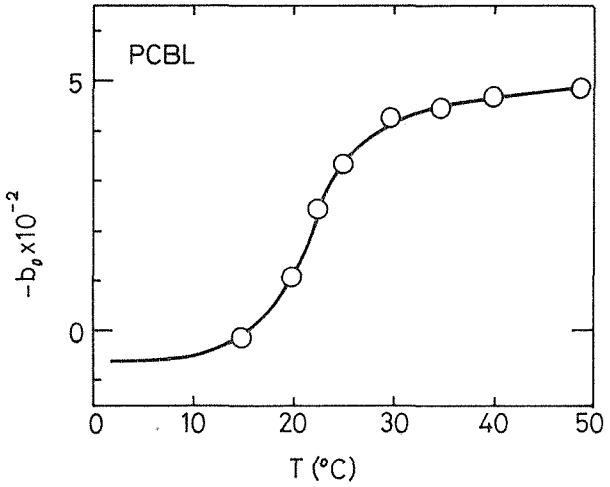
Fig. 6.

enthalpies are of significance. These are: ΔH, the van't Hoff heat of transition, derived from the overall change in helix content of the polypeptide 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 Bragg[12] and of Applequist,[13] ΔH is related to ΔH_{res} by the following equation

$$\Delta H = \frac{\Delta H_{res}}{\sigma^{1/2}} \tag{5}$$



(e) Temperature dependence of b_0 in a 54.5 vol%-DCA-45.5 vol%-DCE mixture for PBGCL copolymer (Sample C-5, 27.0 mol%-BLG).



(f) Temperature dependence of b_0 in a 35.7 vol%-DCA-64.3 vol%-DCE mixture for PCBL (Sample C-6).

Fig. 6.

The parameter σ is designated as the cooperative parameter by Achermann and Neumann.[14]

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_o *vs.* temperature curve using the equation[2,13,15]

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

On the other hand, the value of ΔH_{res} can be directly measured by the calorimetric

technique,[16,17,18] and it has been studied by using various methods such as the temperature dependence of the pH titration[19] and the heat capacity.[20] The values obtained with PBLG by different authors[14,16,17,18,20] scattered from 525 cal/mole to 1000 cal/mole, because ΔH_{res} of PBLG was expected to depend on the molecular weight of PBLG and also on the concentration of PBLG in the solution. While, the value of ΔH_{res} reported with PCBL is extremely rare. Recently, G. Giacometti *et al.*[21] has reported the results of heat of solution measurements of PCBL in DCA-DCE mixture, and obtained $\Delta H_{res}=620\pm40$ cal/mole for PCBL. They also estimated ΔH_{res} with PBLG, PMLG, and PELG in DCA-DCE mixtures at 30°C by measuring the heat of solution. Their experimental results have led to 650 cal/mole for all these three homopolypeptides, and they concluded that the difference of side chain in these polypeptides has no appreciable effect on the transition enthalpy, although it affects the helix stability as judged from the solvent composition at the transition points.

Though the value of ΔH_{res} should directly be measured by calorimetric method described above, it also can be determined approximately by the method proposed by Karasz *et al.*,[22] which based on the determination of heat of fusion from melting point depressions. 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" state. Above T_t, however, these DCA molecules are released and are in normal liquid state. At T_t, the chemical potential of the DCA in the "solid" state equals that of DCA in the liquid state in the DCA-DCE mixture. From the ideal solution approximation the following expressions are derived:

$$\mu_{DCA}-\mu_{DCA}{}^{0} = -RT\ln X_{DCA} \tag{7}$$

$$\mu_{DCA}{}^{s}-\mu_{DCA}{}^{0} = -\Delta H_{DCA}\left(1-\frac{T_t}{T_t{}^{0}}\right) \tag{8}$$

from eq. 7 and 8 we obtain the equation

$$\frac{d(\ln X_{DCA})}{dT_t} = \frac{\Delta H_{DCA}}{RT_t{}^{2}} \tag{9}$$

where $\mu_{DCA}{}^{\circ}$ is the chemical potential of the pure DCA, X_{DCA} is the mole fraction of DCA in the solvent mixture, $T_t{}^{\circ}$ is the transition temperature of the polypeptide in DCA in the absence of DCE, and ΔH_{DCA} is the overall heat, per mole of DCA, associated with the transition of "solid" DCA.

Eq. 9 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 assumed 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_t of the polypeptide. Hence ΔH_{DCA} can be identified with the enthalpy of transition ΔH_{res}. Accordingly,

$$\frac{d(\ln X_{DCA})}{dT_t} = \frac{\Delta H_{res}}{RT_t{}^{2}} \tag{10}$$

Now we will refer to the thermal transition curves of the copolypeptide C-4(45.7 mol%-BLG–54.3 mol%-CBL) in various DCA-DCE mixtures(see Figure 7). The

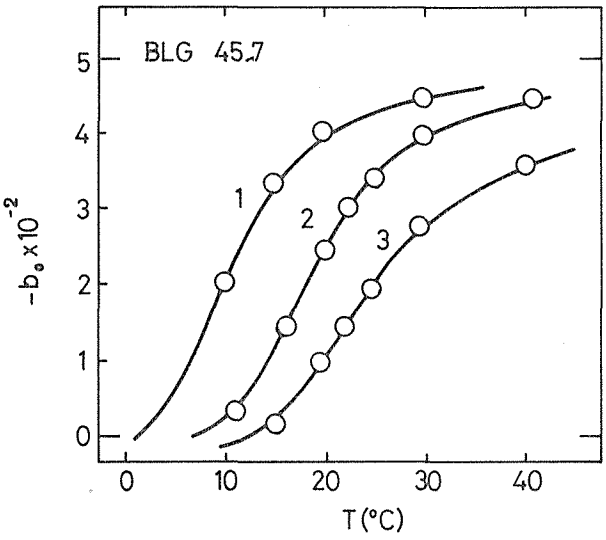


Fig. 7. Temperature dependence of b_0 in DCA-DCE mixtures with various solvent compositions for PBGCL copolypeptide (Sample C-4, 45.7 mol%-BLG); 1) 58.8 vol%-DCA-41.2 vol%-DCE, 2) 61.5 vol%-DCA-38.5 vol%-DCE, and 3) 64.3 vol%-DCA-35.7 vol%-DCE mixture.

sharpness of transition was slightly different from case to case, and the value becomes lower as the DCA-mol% in the DCA-DCE mixture increases. The ΔH values were shown in Table 3. We estimate ΔH and ΔH_{res} by the use of eq. 6 and 10, respectively, for PBGCL (C-4) in DCA-DCE mixtures. The values of σ can be calculated from eq. 5. ΔH_{res} was estimated from eq. 10, showing the relation between the transition temperature T_t and the logarithm of the mole fraction of DCA in the mixture. The value of ΔH_{res} thus obtained was about 700 ± 100 cal/mole on a residue basis. Previously, we have reported that the values of ΔH_{res} for PBLG and PCBL in DCA-DCE mixtures are 930 ± 100 cal/mole[2] and 850 ± 100 cal/mole,[23] respectively. Accordingly, the value of ΔH_{res} for PBGCL copolypeptide (45.7 mol%-BLG) is smaller than those of both homopolypeptides, PBLG and PCBL, but the difference is not so large. We can point out that the value of ΔH_{res} for PBLG obtained by the melting-point depression method, exceeds that found calorimetrically in all cases, though agreement between the former and the result of Ackermann and Neumann[14] ($\Delta H_{res}=950\pm20$ cal/mole) is probably within experimental error. The

Table 3. Composition of Solvent Mixture DCA-DCE, Transition Temperature T_t, ΔH and ΔH_{res} for Copolypeptide C-4 (45.7 mol%-BLG-54.3 mol%-CBL)

No.	DCA (vol%)	Trans. Temp. (T_t(°C)	ΔH (kcal/mol)	ΔH_{res} (cal/mol)
1	58.8	13	25± 5	750±100
2	61.5	22	25± 5	700±100
3	64.3	32	20± 5	650±100

differences between them probably may arise from that eq. 10 is an approximation in that it involves the molar concentration rather than activity, and that imbalance in number of DCA molecules bound to polypeptide coil and number of peptide residues present may be considered. Though our treatment using eq. 10 is regarded as a limiting case, we can assume that, judging from the ΔH_{res} values obtained above, the side chains effect is mainly on the entropy variation in the idealized helix-coil process for a helix with solvated side chains but without any further noticeable solvent interaction with the polypeptide backbone.

Finally the value of σ for PBGCL copolypeptide(45.7 mol%-BLG) was calculated from eq. 5, which led to $\sigma=8\times10^{-4}$. This value is rather higher than those ($\sigma=1.0\times10^{-4}$) of component homopolypeptides PBLG and PCBL. This fact suggests that the transition of the present copolypeptides is performed less cooperatively than that of the corresponding homopolypeptides.

While, Roig *et al.*[4] studied experimentally PBGCL copolymers synthesized with sodium methoxide initiator in dioxane, and analyzed the results in terms of Lifson and Roig's theory.[24] They concluded that the cooperativity parameter changed linearly with polymer and solvent compositions, whereas the heat of the transition, ΔH_{res}, showed a very pronounced minimum as a function of polymer composition. They thought that the reason for the minimum was the influence of the side chains, since they are not taken into account in the theory, and that the influence of the side chains could be due to the high packing of one of them, in this case CBL, because of the presence of amide bond in CBL.

Our results for ΔH in Table 2 are essentially the same behavior as that of Roigs', but if we tentatively adopt the same σ value for the copolypeptide(45.7 mol%-BLG) as found by Roig, the ΔH_{res} value calculated becomes $\Delta H_{res}=350$ cal/mol.

As both methods to obtain ΔH_{res}, *i.e.*, the melting-point depression method and Roig's method,[4] are indirect and involve various approximations, we must measure the value of ΔH_{res} calorimetrically to discuss which is more accurate.

Giacometti *et al.*[21] also have reported results of heat of solution measurements on copolymers of CBL with L-phenylalanine (PA) in mixtures of DCA-DCE. In their data, the values of the heat of transition for PCBL and its PA copolymers, ΔH_{res}, are all practically identical and equal to the values found for the polyglutamates. Such result may suggest a rather notable insensitivity of this quantity on the nature of the side chain.

In summarizing our results, we point out that ΔH derived from the overall change in helical content in the copolypeptides as a function of temperature showed a pronounced minimum as a function of copolymer composition, and that ΔH_{res} also showed a minimum value with respect to copolymer composition.

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