

Effects of Temperature on Molecular Conformations of Poly- γ -methyl-L-glutamate in Solvents

Akio NAKAJIMA and Toshio HAYASHI*

Received March 27, 1968

Change in molecular conformations of poly- γ -methyl-L-glutamate (PMLG) in dichloroacetic acid (DCA), *m*-cresol, dichloroacetic acid/1,2-dichloroethane (DCE), and trichloroacetic acid/acetic acid was studied as a function of temperature by optical rotatory dispersion measurements at 10° to 50°C, and by intrinsic viscosity measurements at 20° to 60°C. The results obtained were compared with those for poly- γ -benzyl-L-glutamate (PBLG). According to the experiments, the following conclusions were obtained. PMLG, in DCA/DCE and in trichloroacetic acid/acetic acid systems, transforms from coil to helix with increasing temperature by the inverse transition mechanism as found for PBLG in DCA/DCE mixture. The enthalpy of transition ΔH for PMLG estimated in DCA/DCE system is 75 ± 10 Kcal/mole and is considerably small compared with the value, $95 \sim 110 \pm 15$ Kcal/mole, for PBLG. The helix stability of PMLG is lower than that of PBLG and it is pointed out that the number of residues co-operatively contributes to transformation is smaller for PMLG (88 residues) than for PBLG (100~110 residues).

INTRODUCTION

In our previous papers^{1,2)}, we have reported on the molecular conformations of poly- γ -methyl-L-glutamate (PMLG) in various solvents, such as dichloroacetic acid (DCA), trifluoroacetic acid (TFA), *m*-cresol, and some mixed solvents. In the present study, we will concern with the influence of temperature upon the molecular conformations of PMLG in various solvent systems.

It is known that the conformation of proteins, nucleic acids, and synthetic polypeptides in solvent is generally transformed from helix to coil with increasing temperature through intermediate stages called interrupted helix. For some polypeptides, however, it is also known that their molecular conformation is changed, according to the change in external condition^{3~8)}, from coil to helix with increasing temperature. Such inverse transition has been found, for example, in the systems: PBLG in mixtures of DCA and dichloroethane (DCE)^{3~5,7)}, in mixtures of DCA and 1,4-butylene dichloride⁹⁾, in mixtures of DCA and octyl chloride⁹⁾, and in mixtures of DCA and 1-dodecyl chloride⁹⁾; Poly- ϵ -carbobenzoxy-L-lysine (PCBL) in mixture of DCA and chloroform (37:63 by volume)⁹⁾.

Both PBLG and PCBL have some common nature on account of their large side-chain groups. But PCBL is known to undergo transition at a somewhat lower concentration of DCA, the hydrogen-bond rupturing component, in DCA/DCE mixture, if we compare these two polymers with systems having the same transition

* 中島章夫, 林 寿郎: Department of Polymer Chemistry, Kyoto University, Kyoto

temperature T_i . The helix of PCBL is, therefore, considered to be less stable than PBLG, though these two polypeptides are rather similar in other respects including optical properties⁹⁾.

In the case of PMLG, the DCA content in DCA/DCE mixture which gives the same transition temperature as that of PBLG is about 5~8 mole% lower than that of PBLG^{2,10)}. Thus, the helix of PMLG is also regarded to be less stable than that of PBLG.

The present paper deals with experimental results on the variations of optical rotatory dispersion and $[\eta]$ associated with thermally induced coil-helix transition of PMLG in DCA, *m*-cresol, DCA/DCE, and trichloroacetic acid (TCA)/acetic acid (AcOH) mixtures. The results obtained were compared with that for PBLG. In particular, the enthalpy of transition ΔH of both polymers in DCA/DCE system was estimated and discussed with respect to helix stability of each polymer.

EXPERIMENTAL

1) polypeptide and Solvent

The monomer, *N*-carboxy- γ -methyl-L-glutamate anhydride (NCA), was prepared by the method proposed by Blout and Karson²²⁾ and purified by repeated recrystallization from an ethyl acetate solution with the addition of petroleum ether. Polymerization of the monomer was carried out at 20°~25°C in mixed solvents of dioxane and methylene chloride of various compositions, in the presence of triethylamine (TEA) as an initiator. Samples of different molecular weights were prepared by varying the concentrations of NCA and TEA in the system. The polypeptide formed was precipitated in a large amount of cold methanol, and dried under reduced pressure at 40°~50°C. The molecular weights of these PMLG were estimated from the limiting viscosity number $[\eta]$ in DCA at 25°C, by using the viscosity equation for PBLG obtained by Doty *et al.*¹¹⁾. The estimated values of degree of polymerization P were 5590, 3700, 2100, and 1120. It should be noted that the molecular weight *vs.* $[\eta]$ relation for PMLG has not yet been known, so the molecular weights thus obtained are just approximated ones. PBLGs were also prepared by the same manner as that mentioned above, and their P values were 3650, 2560, and 1550.

All the solvents used for the preparation of samples were purified according to the standard procedures. DCA (Nakarai Chem. Co., Extra pure Grade) and *m*-cresol (Nakarai Chem. Co., Guaranteed reagent Grade) were vacuum-distilled twice just before using. Other solvents (Nakarai Chem. Co., Guaranteed reagent Grade) were used without further purification.

In the previous paper²⁾, we have found that *m*-cresol, DCE, and acetic acid were "helix solvents", while DCA and TCA were "coil solvents" for both PMLG and PBLG at room temperature.

2) Measurements

Optical rotatory dispersions in a temperature range from 10° to 50°C were measured with a Yanagimoto OR-100 type Spectro-Polarimeter using a tungsten lamp as light source. The wave lengths used covers from 330 to 600 $m\mu$. The concentration of polypeptide solutions was 0.5 g/dl with all of these measurements.

The solvent systems used were *m*-cresol, DCA, DCA/DCE (60~80 mole% DCA), and TCA/AcOH (60~72 mole% TCA). The intrinsic viscosity $[\eta]$ was determined as a function of temperature in the range from 20° to 65°C, using the Ubbelohde type capillary viscometers.

RESULTS AND DISCUSSION

Moffitt and Yang¹²⁾ have shown the following equation for optical rotatory dispersion of polypeptides

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

where a_0 is a constant which may be expected to vary with the nature of the side chain of polypeptide and to be dependent on solvent effects, whereas b_0 and λ_0 are the constants characteristic of the helix; M_0 is the molecular weight per peptide residue, n the refractive index of the solvent and λ the wave length ($m\mu$). In our previous analysis of the dispersion data^{1,2)} on PMLG and PBLG in some solvents where the helical conformation is stable, we obtained $b_0 = -600$ and $\lambda_0 = 212 m\mu$.

1) Behavior in DCA and in *m*-Cresol

Figure 1 shows the temperature dependence of b_0 of PMLG in DCA, and in *m*-cresol, compared with the results of PBLG in the same solvent systems. The b_0 value of PMLG in *m*-cresol is -540 , while that of PBLG in *m*-cresol is -600 in

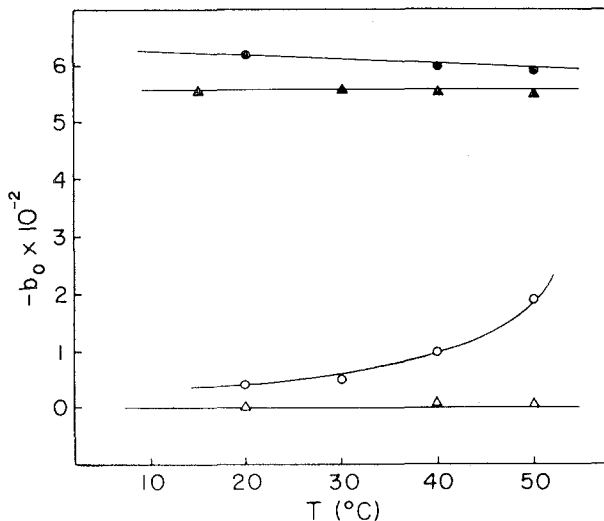


Fig. 1. Temperature dependence of b_0 of PMLG ($P=2100$) in *m*-cresol ($\blacktriangle$) and DCA ($\triangle$), and of PBLG ($P=2560$) in *m*-cresol ($\bullet$) and DCA ($\circ$).

a range of temperature from 20° to 50°C. These two values are approximately constant within the temperature range measured. Therefore, we may regard that the molecular conformation of PMLG and PBLG in *m*-cresol is helix in these temperature range, and that the breakage of the intramolecular hydrogen bonds should not have occurred.

Figure 2 shows the temperature dependence of $[\eta]$. The $[\eta]$ values of both PMLG and PBLG in *m*-cresol are large, indicating the presence of helix conformations, but these values slightly decrease monotonically with increasing temperature. Such result may suggest that the hydrodynamic volume of the polymer in the solvent decreases owing to the diminution of solvation with increasing temperature.

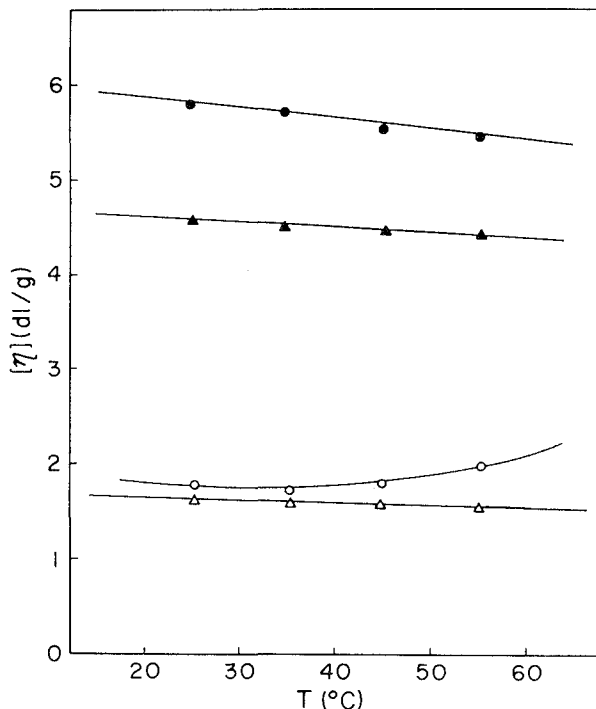


Fig. 2. Temperature dependence of $[\eta]$ of PMLG ($P=2100$) in *m*-cresol (▲) and DCA (△), and of PBLG ($P=1550$) in *m*-cresol (●) and DCA (○).

In DCA, as obvious from Figs. 1 and 2, the values of b_0 and $[\eta]$ for PBLG increase with increasing temperature. This may suggest that PBLG in DCA undergoes a transition from coil to helix when the temperature is raised, *i.e.* the inverse transition takes place. However, in the case of PMLG, the molecular conformation in DCA is coil and does not suffer a transition from coil to helix at least in the range of temperature from 20° to 50°C, though the same behavior as PBLG may be expected in DCA at higher temperature range than that used here.

2) Behavior in DCA/DCE Mixed Solvent Systems

As mentioned above, PBLG takes helical and coiled conformations at room temperature in DCE and DCA, respectively, but partly helical conformation at higher temperatures in DCA.

Now we will concern with the conformational behavior of PMLG accompanied with the change in temperature. Figures 3 and 4 show the temperature dependence of b_0 of PMLG in DCA/DCE mixtures, and that of $[\eta]$ of the same polypeptide in the same mixed solvents, respectively. On the other hand, Fig. 5 shows

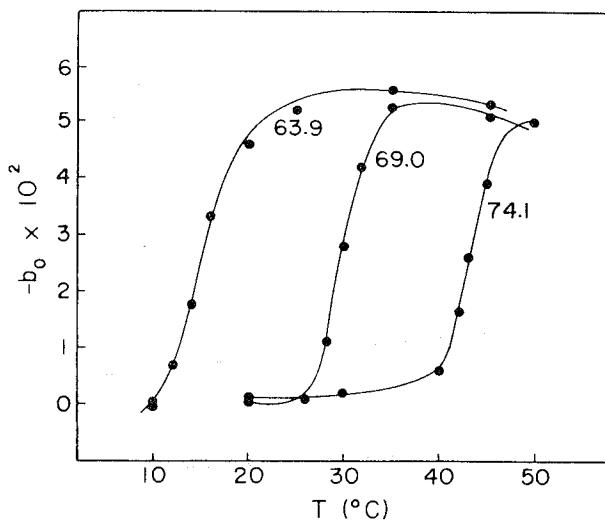


Fig. 3. Temperature dependence of b_0 of PMLG ($P=3700$) in DCA/DCE mixtures. Composition of solvent mixtures is indicated as mole percent of DCA.

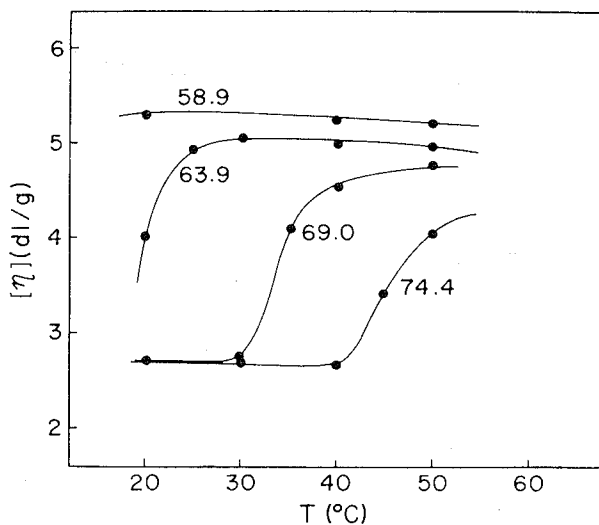


Fig. 4. Temperature dependence of $[\eta]$ (dl/g) of PMLG ($P=3700$) in DCA/DCE mixtures.

the temperature dependence of b_0 of PBLG in DCA/DCE mixtures. From these results, we can see that PMLG in DCA/DCE mixtures transforms from coil to helix with increasing temperature by such an inverse transition mechanism as found for PBLG in DCA/DCE mixtures. PMLG undergoes a coil-to-helix transition at a lower concentration of DCA in DCA/DCE mixtures than does PBLG under the condition which gives the same transition temperature. Namely, the helix of PBLG is considered to be more stable than that of PMLG.

Comparing the data of Fig. 3 with that of Fig. 4, we found that an appreciable change in $[\eta]$ does not occur yet at the transition temperature T_i determined from the b_0 vs. temperature curve and that the $[\eta]$ value at the transition temper-

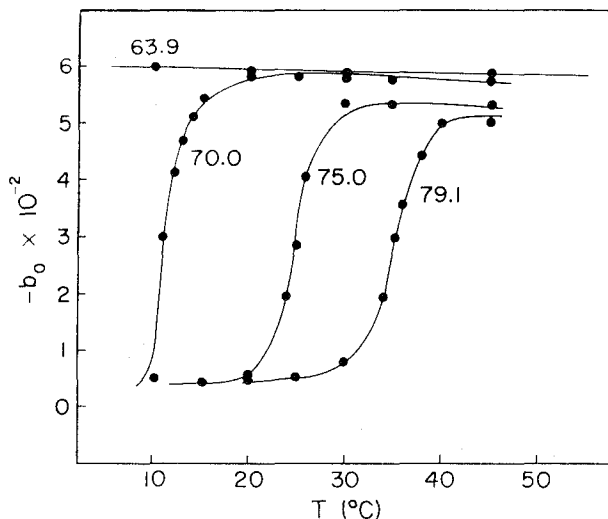


Fig. 5. Temperature dependence of b_0 of PBLG ($P=3650$) in DCA/DCE mixtures.

ature is only slightly larger than the value which corresponds to coil conformation. An appreciable change in $[\eta]$ appears only after the helical content α exceeds about 0.8. Figure 6 shows the relation between the intrinsic viscosity $[\eta]$ and the helical content α , which is estimated from the data in rotatory dispersion measurements, for PMLG in DCA/DCE mixtures of different compositions. Similar result has been reported for PBLG by Fujita *et al.*¹⁸⁾. They pointed out that the value of $[\eta]$ at an α value as high as 0.95 is even considerably lower than that expected for perfectly helical state.

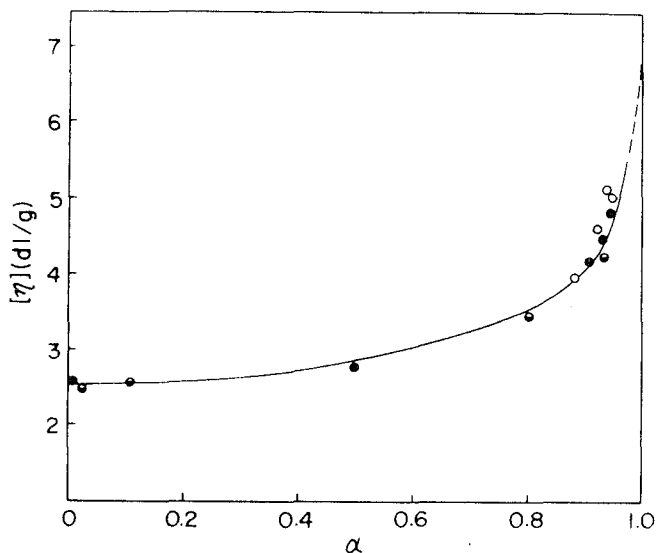


Fig. 6. Functional relations between intrinsic viscosity $[\eta]$ and helix content α for PMLG ($P=3700$) in DCA/DCE mixtures of different compositions. Open circles; 63.9:36.1 mixtures; filled circles, 69.0:31.0 mixtures; half-filled circles, 74.1:25.9 mixtures.

3) Behavior in TCA/AcOH Mixed Solvent Systems

In a previous study²⁾, it has been reported that PMLG undergoes transition in TCA/AcOH mixtures at 25°C at a composition of about 60 mole% TCA. Therefore, in the present work, we discuss temperature dependence of the molecular conformation of PMLG in TCA/AcOH mixtures containing 60 to 70 mole% TCA. Figures 7 and 8 show the results on the temperature dependence of b_0 and $[\eta]$,

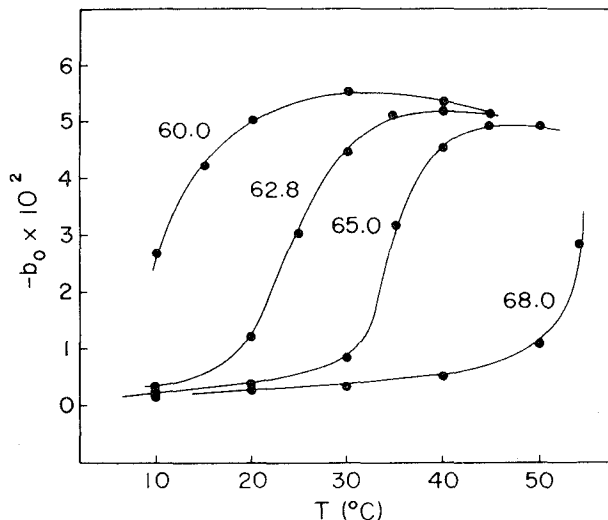


Fig. 7. Temperature dependence of b_0 of PMLG ($P=3700$) in TCA/AcOH mixtures.

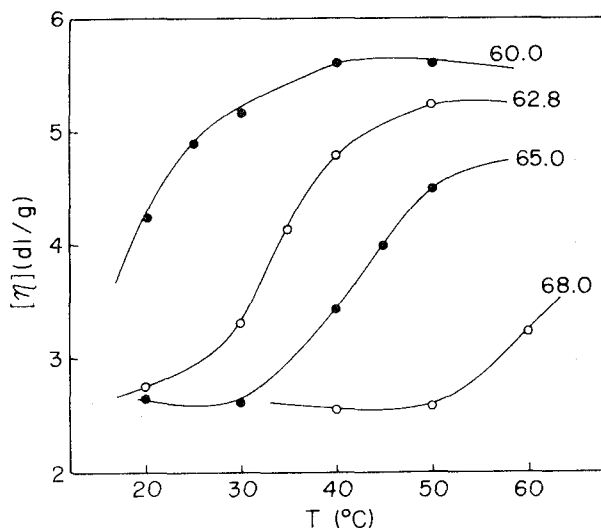


Fig. 8. Temperature dependence of $[\eta]$ (dl/g) of PMLG ($P=3700$) in TCA/AcOH mixtures.

respectively, of PMLG in TCA/AcOH mixtures. While, Figures 9 and 10 indicate the referential data on PBLG in TCA/AcOH mixtures. It is clear from these results that both PMLG and PBLG in adequate TCA/AcOH mixtures undergo a transition from coil to helix as the temperature is raised (inverse transition).

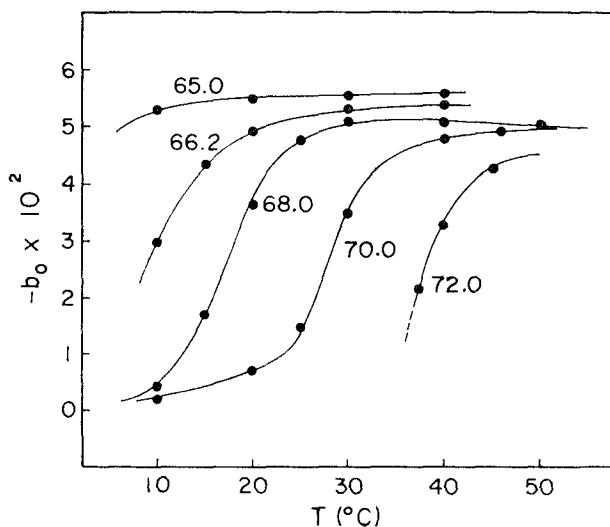


Fig. 9. Temperature dependence of b_0 of PBLG ($P=3650$) in TCA/AcOH mixtures.

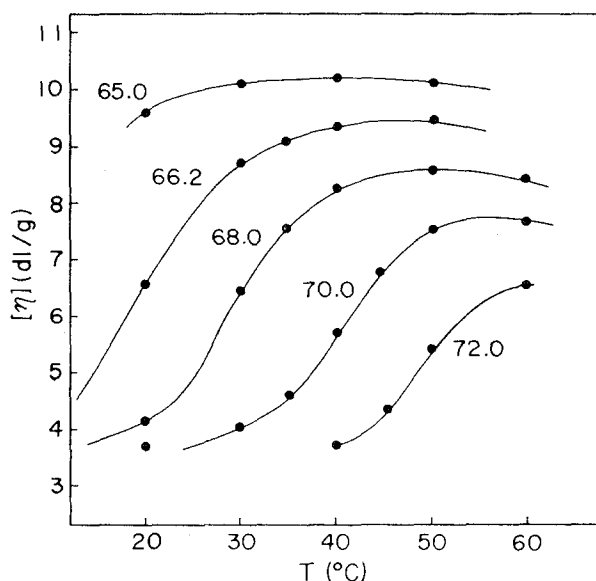


Fig. 10. Temperature dependence of $[\eta]$ (dl/g) of PBLG ($P=3650$) in TCA/AcOH mixtures.

Figure 11 shows the relation between the solvent composition and transition temperature T_i for PMLG and PBLG both in DCA/DCE and TCA/AcOH mixtures. Curves for DCA/DCE systems are concave above, while those for TCA/AcOH systems are convex above. It may be supposed that the difference in the shape of the curves between these two solvent systems comes from that DCE in DCA/DCE mixtures acts merely as an inert diluent, *i.e.* DCE does not interact with the polypeptides, but AcOH in TCA/AcOH mixtures is assumed to interact with the polymers. Later, we will discuss in detail the conformational behavior of PMLG in DCA/DCE.

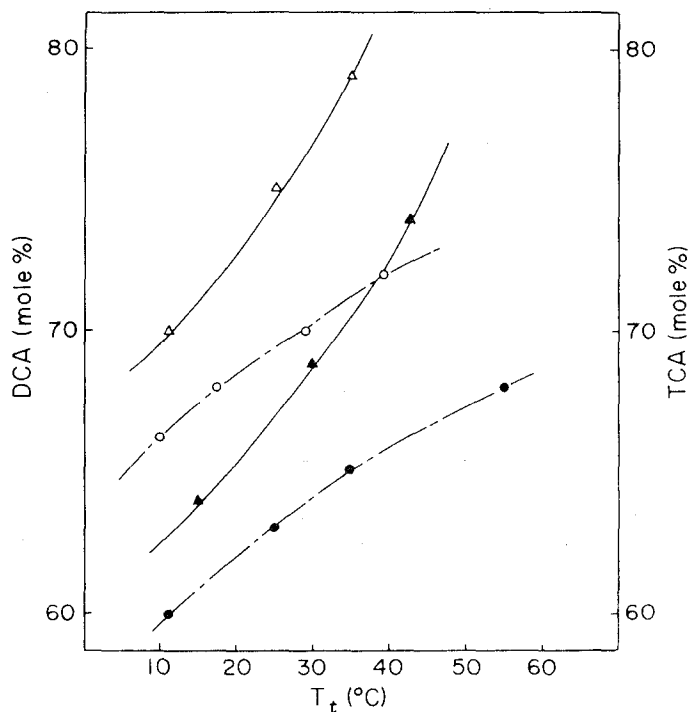


Fig. 11. Transition temperatures of PMLG ($P=3700$) in DCA/DCE mixtures ($\blacktriangle$) and TCA/AcOH mixtures ($\bullet$), and of PBLG ($P=3650$) in DCA/DCE mixtures ($\triangle$) and TCA/AcOH mixtures ($\circ$).

4) Effect of Molecular Weight on T_t

To discuss the effect of molecular weight on transition temperature T_t of PMLG, change in b_0 at constant solvent composition was measured as a function of temperature. Figures 12 and 13 show the data for PMLG in DCA/DCE mixture

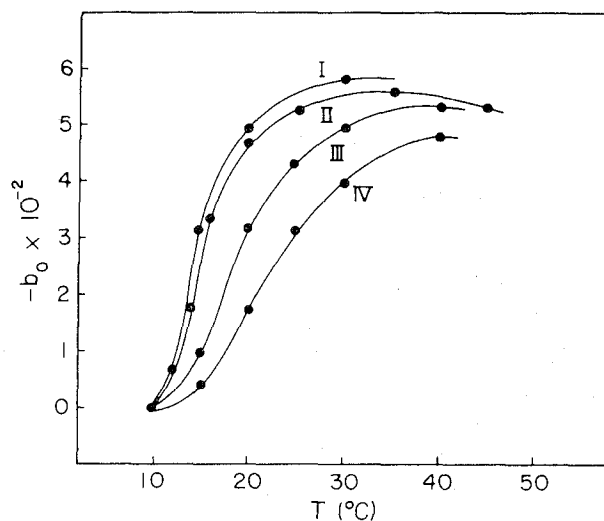


Fig. 12. Temperature dependence of b_0 for PMLG in DCA/DCE mixtures (63.9 mole% DCA). $P=5590$ (I), 3700 (II), 2100 (III), 1120 (IV).

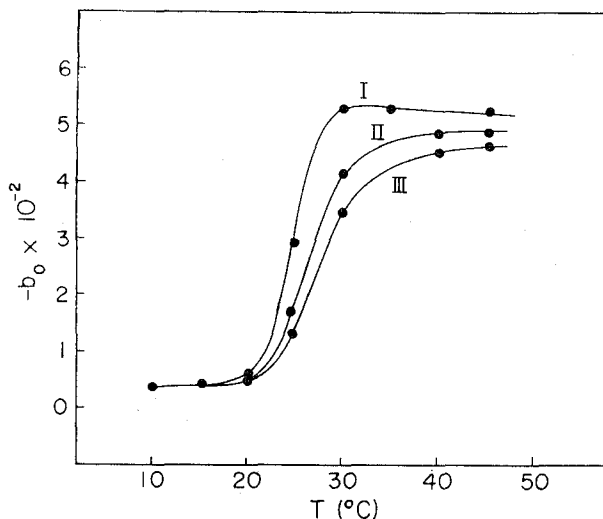


Fig. 13. Temperature dependence of b_0 for PBLG in DCA/DCE mixtures (75.0 mole% DCA). $P=3650$ (I), 2560 (II), 1550 (III).

(63.9 mole% DCA) and PBLG in DCA/DCE mixture (75.0 mole% DCA), respectively. It is clear from these data that the transition temperature shifts toward lower temperature and, at the same time, the transition becomes sharper, as the molecular weight of the polypeptide becomes higher. Taking into consideration that the transition is an inverse transition, these results seem to be theoretically reasonable. The similar result was also reported for PBLG by Fujita *et al.*^{17).}

5) Mechanism of the Inverse Transition

According to the experimental results, PMLG, in DCA/DCE and TCA/AcOH systems, transform from coil to helix with increasing temperature in the same manner as PBLG in DCA/DCE and TCA/AcOH systems. In such transition, not only the conformational change of the polymer, but also the change of solvent interactions may play important roles. In other words, we can say that the characteristics of solvent would give important effects on these transitions. In DCA/DCE system, DCA is important for the transition, but DCE may act only as an inert diluent. At lower temperature, there exist hydrogen-bonds between DCA and peptide residues which are in coiled conformation, while at higher temperature, these hydrogen bonds are broken and the peptide residues form hydrogen bond intramolecularly in a manner to form helix whereby the DCA molecules may form dimers by themselves. On the other hand, in TCA/AcOH system, TCA may perform similar role as DCA in DCA/DCE, but the role of AcOH in TCA/AcOH system may not quite the same as that of DCE in DCA/DCE system. We may expect some interaction between TCA and AcOH, therefore, the effect of TCA on the coil-helix transition of polypeptide in this mixed solvent system may be strengthened by the presence of AcOH. This trend is obvious from the fact²⁾ that the concentration of DCA in DCA/DCE mixture which gives the transition temperature at 25°C is 70 mole% whereas that of TCA in TCA/AcOH mixture which gives the same transition temperature is only 60 mole% for PMLG of a definite molecular weight.

Figure 14 illustrates scheme of the transition of polypeptide in DCA/DCE system. In Fig. 14, the symbol S means DCA, and a dotted line represents a hydrogen bond. One peptide residue forms on the average two hydrogen bonds with DCA molecules when it is in coiled conformation, so we designate the product as C-2S. The symbol H represents a peptide residue in helical conformation, and S₂ dimerized DCA. There is one intramolecular hydrogen bond per each peptide residue when the chain is in helical conformation and one hydrogen bond in a S₂, hence, it may be assumed that the total number of hydrogen bonds on an average is not changed throughout the transition.

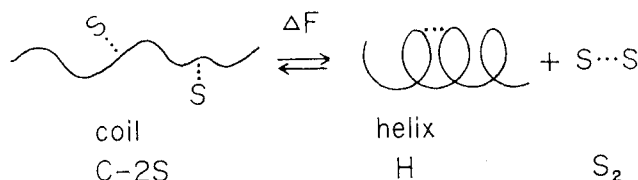


Fig. 14. Inverse transition.

Therefore, the thermodynamic quantity, such as the changes in the free energy ΔF of the transition, includes the net change in the quantities related to the hydrogen bonds as shown in the figure. Thus, the reaction is given by the following equation



where K is the equilibrium constant of the reaction. Hence, the free energy of transition ΔF is shown by the equation,

$$\Delta F = -RT \ln K = -RT \ln \frac{[\text{H}][\text{S}_2]}{[\text{C-2S}]} \quad (3)$$

where, $[\text{H}]$, $[\text{S}_2]$, and $[\text{C-2S}]$ represent the molar concentrations of the peptide residue in helix conformation, the dimerized DCA, and the peptide residue bonded with DCA in coil conformation, respectively, and T is the temperature.

Further, the following equations are introduced



where K_1 and K_2 are the equilibrium constants. The fractional helix content α is defined by the following equation,

$$\alpha = \frac{[\text{H}]}{[\text{C}] + [\text{H}]} \quad (6)$$

where $[\text{H}]$ and $[\text{C}]$ represent the concentrations of peptide residues in helix and coil conformation, respectively.

By combining Eqs. (3), (4), (5), and (6), we obtain the equation.

$$\alpha = \left(1 + \frac{K_2}{K_1} \exp \frac{\Delta F}{RT}\right)^{-1} \quad (7)$$

Thermodynamically the enthalpy of transition ΔH is related to the free energy of transition ΔF by $d(\Delta F/T)/dT = -\Delta H/T^2$, hence, by differentiating Eq. (7) with respect to T , we derive the following equation.

$$\frac{d\alpha}{dT} = \frac{(K_2/K_1) \exp(\Delta F/RT)}{[1 + (K_2/K_1) \exp(\Delta F/RT)]^2} \cdot \frac{\Delta H}{RT^2} \quad (8)$$

At the transition temperature T_i , $\Delta F=0$ and $\alpha=1/2$, therefore, $(K_2/K_1)=1$ is obtained from Eq. (7). Using these relations in Eq. (8), we obtain Eq. (9) at the transition temperature T_i .

$$\left(\frac{d\alpha}{dT}\right)_{T_i} = \frac{\Delta H}{4RT_i^2} \quad (9)$$

On the other hand, it is known that α is linearly proportional to the change in optical rotatory dispersion parameter b_0 and can therefore be estimated from the equation,

$$\alpha = \frac{b_0 - b_{0,c}}{b_{0,h} - b_{0,c}} \quad (10)$$

where b_0 is the value of parameter at a temperature T in the transition region, $b_{0,c}$ and $b_{0,h}$ are those characteristic for perfect coil and perfect helix, respectively. Combining Eqs. (9) and (10), we obtain the equation

$$\left(\frac{d\alpha}{dT}\right)_{T_i} = \frac{1}{b_{0,h} - b_{0,c}} \left(\frac{db_0}{dT}\right)_{T_i} = \frac{\Delta H}{4RT_i^2} \quad (11)$$

By the use of Eq. (11), we can evaluate the enthalpy of transition ΔH experimentally. Here, it should be noticed that the value of the enthalpy of transition ΔH calculated from Eq. (11) does not express the heat associated with the transfer of one peptide residue from the randomly coiled conformation to the helical conformation, but is related to that of one polypeptide as a whole. Thus, if the polypeptide, whose degree of polymerization is given by P , is composed perfectly of residues in helix conformation, then the enthalpy of transition per peptide residue is indicated by $\Delta H/P$.

In the formulation of Zimm and Bragg¹⁴⁾ and of Applequist¹⁵⁾, ΔH of Eq. (11) is related to the enthalpy of formation ΔH_{res} of intramolecular hydrogen bonds per residue, *i.e.* the transition enthalpy per peptide residue associated with the transfer from coil to helix, by the following equation

$$\Delta H = \Delta H_{res}/\sigma^{1/2} \quad (12)$$

where, σ is the co-operative parameter related to the average length of the helical sequences at T_i . At the limiting case in which the whole residues are co-operative, $\Delta H_{res} = \Delta H/P$ and, hence, σ is equal to $1/P^2$. However, the σ values experimentally reported by Doty *et al.*²⁰⁾ are in a range of 1×10^{-4} to 2×10^{-4} for PBLG-DCA-DCE system. According to these numerical values of σ , it is pointed out that the number of residues co-operatively contributes to transformation is 100~70 residues.

Though the value of ΔH_{res} can directly be measured by calorimetric technique^{6-8,13,16)}, it also can be determined by the method proposed by Karasz *et al.*⁹⁾, 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 temperature below T_i , and are therefore in a "solid" phase. Above T_i , however, these DCA molecules are released and in normal liquid state. At T_i , the chemical potential μ_{DCA}^s of the DCA in the "solid" state equals to that μ_{DCA}^l of DCA in the liquid state in DCA/DCE mixture. The ideal solution approximation derives the following expressions

$$\mu_{\text{DCA}}^1 - \mu_{\text{DCA}}^0 = -RT \ln x_{\text{DCA}}$$

$$\mu_{\text{DCA}}^s - \mu_{\text{DCA}}^0 = -\Delta H_{\text{DCA}} (1 - T_t/T_t^0)$$

from which we obtain the equation

$$d(\ln x_{\text{DCA}})/dT_t = \Delta H_{\text{DCA}}/RT_t^2 \quad (13)$$

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

Equation (13) was derived for the transition of DCA from "solid" state to "liquid" state. Now the total number of hydrogen bonds is, as above mentioned, not changed throughout the transition, 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_t of the polypeptide, hence, ΔH_{DCA} can be identified with the enthalpy of transition ΔH_{res} . Accordingly,

$$d(\ln x_{\text{DCA}})/dT_t = \Delta H_{\text{res}}/RT_t^2 \quad (14)$$

Now we estimate ΔH and ΔH_{res} by the use of Eqs. (11) and (14), respectively, for PMLG and PBLG in DCA/DCE mixtures. Table 1 is a summary of these data. In these calculations, the numerical values, $b_{0,c}=0$ and $b_{0,h}=-600$, were used for estimating ΔH . ΔH_{res} was estimated from Fig. 15, which showed the relation between transition temperature T_t and the logarithm of the mole fraction of DCA in the mixture. The relation $\Delta S_{\text{res}} = \Delta H_{\text{res}}/T_t$ was used to estimate the conforma-

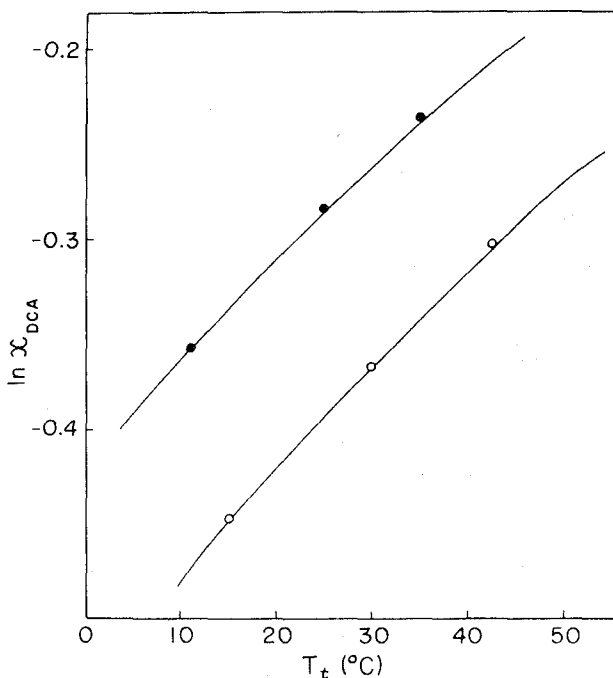


Fig. 15. Transition temperatures as a function of solvent composition x_{DCA} (=mole fraction of DCA), for (●) PMLG ($P=3700$), and (○) PBLG ($P=3650$) in DCA-DCE system.

tional entropy change per peptide residue ΔS_{res} . Numerical values of σ were also calculated by Eq. (12). These results were included in Table 1.

Table 1. Enthalpy of transition ΔH , and enthalpy of helix formation per residue ΔH_{res} , for PMLG and PBLG in DCA-DCE mixtures.

Polypeptide	P	x_{DCA}	$T_t(^{\circ}\text{K})$	ΔH (Kcal/mole)	ΔH_{res} (cal/mole)	ΔS_{res} (e.u)	$\sigma \times 10^4$
PMLG	3700	0.64	288	75 ± 10	955 ± 100	3.3 ± 0.3	1.3
	3700	0.69	303	75 ± 10	945 ± 100	3.2 ± 0.3	1.3
	3700	0.74	315.5	70 ± 10	935 ± 100	3.0 ± 0.3	1.4
PBLG	3650	0.70	284	110 ± 15	935 ± 100	3.4 ± 0.3	0.75
	3650	0.75	298	100 ± 15	930 ± 100	3.2 ± 0.3	0.9
	3650	0.79	308	95 ± 15	925 ± 100	3.1 ± 0.3	1.0
	2560	0.75	300	95 ± 15	—	—	—
	1550	0.75	301.5	90 ± 15	—	—	—

It is pointed out from Table 1 that ΔH_{res} values of PMLG are about the same as those of PBLG, while ΔH values of PMLG are less than those of PBLG. ΔH_{res} is concerned only with the intramolecular hydrogen bonds in the backbone of polypeptide, and so we may assume that the substituted side group of the polypeptide may not affect largely on ΔH_{res} , whether it is methyl or benzyl group. Thus, the experimental results on ΔH_{res} are quite reasonable. Further, these experimental values closely agree with the values reported by Zimm⁽¹⁴⁾ and Lifson⁽²¹⁾.

On the other hand, the values of ΔH for PBLG are larger than that of PMLG, and accordingly σ -values of PBLG are less than those of PMLG. In ΔH the effect of co-operativeness should be included and so the conformation and structure of side chain group may largely affect on the value of ΔH . The result that ΔH of PBLG is larger than that of PMLG is to suggest that the helix of PBLG is considered to be more stable than that of PMLG because of the benzyl group which is more bulky than methyl group, and further that the transition is performed more co-operatively in the case of PBLG than in that of PMLG. From $\sigma = 1.3 \times 10^{-4}$ for PMLG, the number of residues which contributes to the transition may be estimated as about 88 residues, while that of PBLG may be estimated as 115~100 residues from $\sigma = 0.75 \times 10^{-4} \sim 1.0 \times 10^{-4}$.

Lastly, we refer to ΔS_{res} . ΔS_{res} is expected to depend on the kind of side chain. But the values of PBLG and PMLG are not largely differ from each other. Though it is not clear at present whether the difference in side chain groups of these two polypeptides could lead to a difference in ΔS_{res} or not, we may propose that the $-\text{CH}_2\text{-CH}_2\text{-CO-O-}$ portion in side chain largely affects on ΔS_{res} but methyl or benzyl which locates far from the backbone hardly affects on ΔS_{res} .

This investigation was supported by a grant from the Ministry of Education.

REFERENCES

- (1) A. Nakajima and T. Hayashi, *Chem. High Polymers (Japan)*, **24**, 230 (1967).
- (2) A. Nakajima and T. Hayashi, *Chem. High Polymers (Japan)*, **24**, 235 (1967).
- (3) P. Doty and J.T. Yang, *J. Am. Chem. Soc.*, **78**, 498 (1956).

- (4) J.A. Schellman, *J. Phys. Chem.*, **62**, 1485 (1958).
- (5) M. Calvin, J. Hermans, Jr., and H.A. Scheraga, *J. Am. Chem. Soc.*, **81**, 5047 (1959).
- (6) F.E. Karasz, J.M. O'Reilly and H.E. Bair, *Biopolymers*, **3**, 241 (1965).
- (7) F.E. Karasz and J.M. O'Reilly, *Biopolymers*, **4**, 1015 (1966).
- (8) F.E. Karasz and J.M. O'Reilly, *Biopolymers*, **5**, 27 (1967).
- (9) G.D. Fasman, M. Idelson and E.R. Blout, *J. Am. Chem. Soc.*, **83**, 709 (1961).
- (10) A. Nakajima, S. Tanaka and T. Hayashi, Reported at 13th Research Meeting of the Society of Polymer Science Japan (1967-7-8, Kobe).
- (11) P. Doty, *J. Am. Chem. Soc.*, **79**, 3961 (1957).
- (12) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).
- (13) T. Ackermann, *Biopolymers*, **5**, 649 (1967).
- (14) B.H. Zimm and J.K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
- (15) J. Applequist, *J. Chem. Phys.*, **38**, 934 (1963).
- (16) F.E. Karasz, *Nature*, **202**, 693 (1964).
- (17) H. Fujita, A. Teramoto and T. Yamashita, *J. Chem. Phys.*, **46**, 1919 (1967).
- (18) H. Fujita, A. Teramoto and K. Nakagawa, *J. Chem. Phys.*, **46**, 4197 (1967).
- (19) R. Sakamoto, Reported at 16th Discussion Meeting of the Society of Polymer Science Japan (1967-10-19, Fukuoka).
- (20) P. Doty, *Proc. Natl. Acad. Sci. (US)*, **45**, 1601 (1959).
- (21) S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961).
- (22) E.R. Blout and R.H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).