

Solution Properties of Synthetic Polypeptides. X. Transition Parameters for Poly(γ -Benzyl-L-glutamate)

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ABSTRACT: Helix-initiation parameter σ and enthalpy change ΔH for helix-coil transition of poly(γ -benzyl-L-glutamate) in mixtures of dichloroacetic acid and ethylene dichloride were determined at fixed solvent conditions from the dependence of helical content of the dissolved polypeptide on its molecular weight. The values of $\sqrt{\sigma}$ obtained were substantially independent of solvent composition and temperature over the range where the substantial portion of the transition occurred, yielding $(0.95 \pm 0.13) \times 10^{-2}$ on the average. This value stood in close agreement with that evaluated from calorimetric measurements by Ackermann and Neumann. On the other hand, ΔH depended slightly on solvent composition and had values between those of Ackermann and Neumann and of Karasz and O'Reilly.

KEY WORDS Helix-Coil Transition / Dilute Polymer Solution / Polypeptide / Helical Content / Helix-Initiation Parameter / Transition Enthalpy /

The conformation of a helix-forming polypeptide molecule in dilute solution is characterized by three parameters N , σ , and u . Here N is the number of monomeric residues in the molecule, σ the helix initiation (or cooperativity) parameter, and u the equilibrium constant for the formation of helix from random coil. Customarily, $\ln u$ is expressed in the form:

$$\ln u = - \frac{1}{RT} (\Delta H - T\Delta S) \quad (1)$$

where T is the absolute temperature, R is the gas constant, and ΔH and ΔS are the changes in enthalpy and entropy per mole of monomeric residues which accompany the formation of intramolecular hydrogen bonds.

Zimm, Doty, and Iso¹ were the first to attempt determining σ , ΔH , and ΔS from experiment. They dealt with data for helical content versus temperature on three samples of poly(γ -benzyl-L-glutamate) (PBLG) in mixtures of dichloroacetic acid (DCA) and ethylene dichloride (EDC) and tried to find a set of values for ΔH , ΔS , and σ which would make the theoretical equation of Zimm and Bragg² fit these data. This method,

which may be referred to as the curve-fitting method, is tedious to carry through and restrictive in that it assumes the parameters ΔH , ΔS , and σ to be independent of temperature in the region where the polypeptide undergoes conformational transition from random coil to helix. In view of these disadvantages of the method of Zimm, *et al.*,¹ we have developed in Part VI³ of this series a new procedure which is free from the above-mentioned restriction.

The purpose of the present paper is to apply it to PBLG in mixtures of DCA and EDC and compare the resulting values for ΔH and σ with those reported on the same systems by previous authors.^{1,4-7}

EXPERIMENTAL

Polypeptide Samples

γ -Benzyl-L-glutamate purchased from the Peptide Center of Osaka University was converted to γ -benzyl-L-glutamate-N-carboxyanhydride (NCA) by the method of Blout and Karlson.⁸ The NCA was purified by repeated crystallization under nitrogen atmosphere first from a chloroform solution and then from an ethyl

acetate solution with *n*-hexane as a precipitant. Polymerization of NCA was carried out at room temperature with dioxane as a solvent and triethylamine as an initiator. In doing this, the concentration of NCA was adjusted to about 5% (wt/vol) and the mole ratio of NCA to initiator to 70. The resulting viscous solution was poured with vigorous stirring into a large volume of ethanol to isolate the polypeptide formed. The polymer obtained was divided into two parts by fractional precipitation from an 0.2% (wt/vol) methylene chloride solution with methanol as a precipitant. The higher-molecular-weight portion, which amounted to 90% of the whole material, was chosen for the present study. This fraction is designated sample E-1 in the subsequent presentation. In addition to this sample, the following three samples were used for the present experiment.

Samples E-3 and A-IV were appropriate middle fractions extracted from our previously prepared samples; one for sample E-3 had been obtained by polymerization with triethylamine as an initiator and one for A-IV with sodium methoxide as an initiator. Sample A-6 was an unfractionated polymer which had been prepared by polymerization in dimethylformamide (DMF) with *n*-butylamine as an initiator.⁹ Kinetic evidence^{9,10} had suggested that this last sample would be nearly monodisperse in molecular weight and so no further fractionation was effected on it.

Each of these samples was precipitated from a methylene chloride solution, and dried further overnight in a vacuum oven at 40°C. The solutions were made up gravimetrically, by effecting the mixing with solvent under nitrogen atmosphere. They were stable at least over ten hours when kept below 40°C.

Solvents

Fractionally distilled triethylamine (bp 89.4°C), chloroform (bp 61.4°C), ethyl acetate (bp 77.3°C), dioxane (bp 102.2°C), and dimethylformamide (bp 41.0–42.0°C (11 mm)) were used. DCA was distilled twice with concentrated sulfuric acid under reduced nitrogen atmosphere, and the fraction boiling at 61–64°C at 3 mmHg was collected and immediately used.

With the aid of a Bausch-Lomb Precision Sugar Refractometer the refractive indices of

various DCA—EDC mixtures at 20, 25, and 30°C and of DMF at 25°C were measured for light of wavelengths 436, 546, and 589 m μ . Densities of these liquids were determined pycnometrically at 25°C. Those of other DCA—EDC mixtures at various temperatures were estimated from these and Yamashita's data.¹¹

Molecular-Weight Determination

The number-average molecular weights \bar{M}_n of the chosen samples of PBLG were determined from osmotic-pressure measurements made at 25°C with DMF as a solvent. Use was made of a Mechrolab Model 502 high-speed osmometer fitted with gel cellophane membranes for higher-molecular-weight samples E-1 and E-3 and with Sartrious Ultrafine Filters of the type Super Dense for lower-molecular-weight samples A-IV and A-6.

The weight-average molecular weights \bar{M}_w of samples E-1 and E-3 were determined by light scattering and those of samples A-IV and A-6 by sedimentation equilibrium, with DMF at 25°C as a solvent. Specific refractive index increments of the polypeptide in DMF at 25°C were 0.121₀ ml/g at 436 m μ and 0.117₁ ml/g at 546 m μ when measured by a differential refractometer of the modified Schulz—Cantow type. Its partial specific volume in the same solvent medium was taken to be 0.78₀ ml/g.⁹ Details of the light-scattering measurements performed will be reported in another paper of this series. The sedimentation equilibrium data were taken with the aid of a Beckman—Spinco Model E ultracentrifuge equipped with the Rayleigh interference optical system and a Kel-F coated 12-mm double-sector cell.

Figure 1 shows the results from osmotic-pressure measurements. Table I summarizes the values obtained for \bar{M}_n , \bar{M}_w , and second virial coefficient A_2 from the above-mentioned experiments. The polypeptide samples chosen were fairly homogeneous with respect to molecular weight when judged from their ratios of \bar{M}_w to \bar{M}_n .

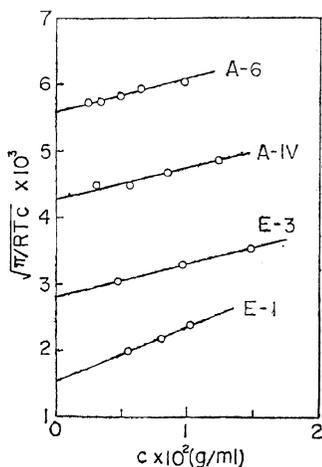
Optical-Rotation Measurements

Data for optical rotation as a function of temperature were obtained by the use of a JASCO Model DIP-SL automatic polarimeter with a jacketed 10-cm cell. Optical rotatory dispersion

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Table I. Numerical data of molecular weight and second virial coefficient for PBLG in DMF at 25°C

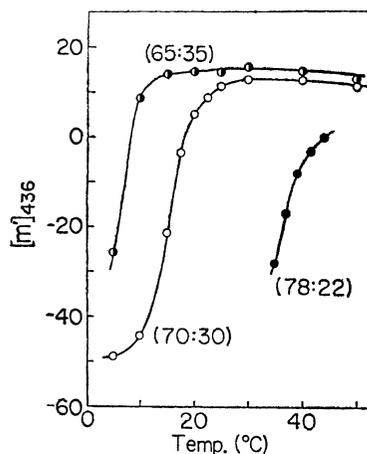
Sample code	Osmotic pressure		Light scattering or sedimentation equilibrium		
	$\bar{M}_n \times 10^{-4}$	$A_2 \times 10^4$, ml mol/g ²	$\bar{M}_{w0} \times 10^{-4}$	$A_2 \times 10^4$, ml mol/g ²	\bar{M}_w/\bar{M}_n
E-1	42.2	2.4 ₄	59.2	2.1 ₈	1.4 ₀
E-3	12.8	2.8 ₀	16.5	2.6 ₇	1.2 ₉
A-IV	5.4 ₉	4.0	6.3 ₅	5.8	1.1 ₆
A-6	3.2 ₀	5.5	3.7 ₀	8.4	1.1 ₆


Figure 1. Plots of $(\pi/RTc)^{1/2}$ vs. concentration c (g/ml) for samples E-1, E-3, A-IV, and A-6 in DMF at 25°C.

(ORD) measurements were performed on a Yanagimoto Model ORD 185 recording spectropolarimeter, and the resulting curves were analyzed in terms of the Moffitt plot¹² (with λ_0 taken to be 212 m μ as usual) to determine the Moffitt parameter b_0 . Mean residue rotations at 436 m μ calculated from the ORD diagrams were compared with the polarimeter values at the corresponding wavelength and external conditions in order to check the accuracy of our ORD apparatus. All data were taken at polypeptide concentrations between 0.5 and 1.0 g/dl, and no effort was made to extrapolate them to infinite dilution.

For refractive indices needed to convert optical rotation to mean residue rotation we used directly measured values or those estimated from them as well as the literature values¹³ from

Sellmeier's equation.


Figure 2. Temperature dependence of mean residue rotation at wavelength 436 m μ , $[m']_{436}$, for sample E-3 in DCA—EDC mixtures. Composition of each DCA—EDC mixture is indicated by volume ratio of DCA to EDC at 25°C.

RESULTS AND DISCUSSION

Estimate of Helical Content from ORD Data

Figure 2 shows typical results from polarimeter measurements on sample E-3 in various DCA—EDC mixtures. The ordinate $[m']_{436}$ represents mean residue rotation at the wavelength of 436 m μ . The compositions of mixed solvents are indicated in terms of volume ratios of DCA to EDC at 25°C. This rule applies to all the subsequent presentations. As will be shown below, $[m']_{436}$ is linearly related to the helical content of the dissolved polypeptide. Thus Figure 2 can be taken to manifest how the helical content of sample E-3 in DCA—EDC mixtures

changes with temperature. It is seen that as the composition of EDC decreases the transition from coil to helix shifts toward higher temperature and becomes more gradual. The two curves with lower DCA contents show broad maxima and $[m']_{436}$ decreases slightly upon further heating. Thus the helical content appears to decrease on the helix side as the temperature is raised. A similar phenomenon was observed by Nakajima and Hayashi¹⁴ on PBLG and poly (γ -methyl-L-glutamate). The reason for this phenomenon is not clear to us, and we wish to leave it for a future investigation. Figure 3

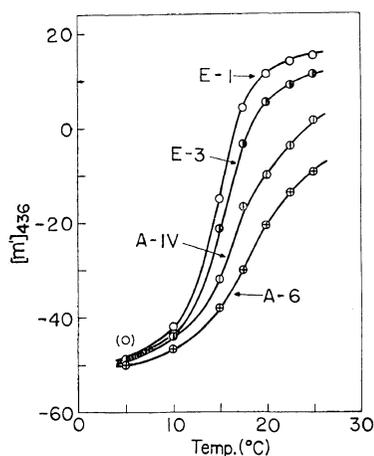


Figure 3. Temperature dependence of $[m']_{436}$ for PBLG in a DCA—EDC (70 : 30) mixture as a function of molecular weight.

shows plots for $[m']_{436}$ vs. temperature in a DCA—EDC (70 : 30) mixture as a function of the molecular weight of PBLG. These results confirm the observations of previous investigators.^{1,14-17}

It is generally accepted that the helical content f_N of a polypeptide molecule is related to the Moffitt parameter b_0 by

$$f_N = \frac{b_0 - b_0^C}{b_0^H - b_0^C} \quad (2)$$

where b_0^C and b_0^H denote the b_0 values for random coil and helix, respectively. The subscript N has been attached to f in order to indicate that the helical content of a polypeptide molecule depends on its degree of polymerization. For eq 2 to be used in practice we must know

the values of b_0^C and b_0^H from appropriate experiment, but this causes a problem of considerable controversy.

First, we assign a value of 45.3 to b_0^C . This is the b_0 value we obtained in pure DCA at 25°C with a PBLG sample of relatively low-molecular weight ($\bar{M}_v = 2.2 \times 10^4$; \bar{M}_v stands for viscosity-average molecular weight). On the other hand, a value of -700 is assigned here to b_0^H , which is the b_0 value obtained in pure EDC at 25°C with another sample of very high molecular weight ($\bar{M}_v = 5.0 \times 10^5$). This assignment is based on the fact that EDC is one of the strongest helix-supporting solvents for PBLG. Our $-b_0$ value with EDC is larger than the reported values which scatter between 635 and 660.^{18,19} Experience indicates that evaluation of b_0 usually cannot escape from an error of a few percent since it resorts to graphical determination of the slope of a plot. Furthermore, factors such as the range of wavelength used for ORD measurements may affect the magnitude of b_0 . Considering these, it does not seem significant to argue about the difference between our $-b_0$ of 700 and the reported 660. In fact, in Part III¹⁵ of this series we had calculated f_N by assuming 660 as the appropriate value for $-b_0^H$.

Although eq 2 offers the most reliable means available at present for the evaluation of helical content of a polypeptide in solution, its use is not convenient in practice because the experimental determination of b_0 is not necessarily simple. This inconvenience can be circumvented, however, if, as has been demonstrated in Part III¹⁵ of this series, there is a unique relation between $[m']_{\lambda}$ at some specified wavelength λ and b_0 for the system considered. This is because the former can be determined more directly and accurately than the latter. We therefore attempted to ascertain the previously established relation for PBLG in DCA—EDC mixtures with additional measurements. The results obtained are shown in Figure 4, together with the previous data of Teramoto, *et al.*¹⁵ Both sets of plotted points fall on a single curve which may be represented approximately by two straight lines intersecting at $-b_0 = 630$ and indicate that in the region of $-b_0$ smaller than 630 there exists a linear relation between $[m']_{436}$

and b_0 , independent of solvent composition and temperature. The branch for $-b_0$ above 630 will not be used for the present analysis, because the data we obtained in this region are too few to conclude that there exists a unique relation between $[m']_{436}$ and b_0 .

Now we substitute $[m']_{436}$ for b_0 in eq 2 with the aid of the solid line shown in Figure 4.

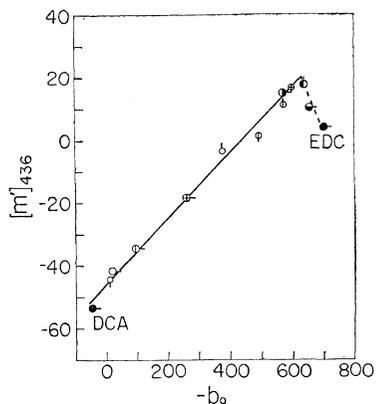


Figure 4. Relationship between $[m']_{436}$ and Mofitt parameter b_0 for PBLG in various DCA—EDC mixtures. \circ , $\bar{M}_v = 1.4 \times 10^4$; \oplus , $\bar{M}_v = 2.2 \times 10^4$, and \oplus , A-6 in DCA—EDC (70 : 30); \ominus , A-6 in DCA—EDC (50 : 50); \odot , E-1 in DCA—EDC (50 : 50); \ominus , $\bar{M}_v = 50 \times 10^4$ in DCA—EDC (3 : 97), and \bullet , in pure DCA and EDC (see text). Small circles indicate the data of Teramoto, *et al.*¹⁵, in DCA—EDC \circ (76 : 24), \oplus DCA—EDC (70 : 30), and DCA—EDC \oplus (64 : 36). The temperatures at which the data were taken are indicated by pips: \circ , 30°C; \square , 25°C; and \triangle , 20°C.

The result is

$$f_N = ([m']_{436} + 50.7)/78.4 \quad (3)$$

In what follows, we shall convert all the measured values of $[m']_{436}$ to f_N in terms of this relation. In this connection, we must remark that eq 3 differs appreciably from the relation between the helical content and mean residue rotation which had been employed by Zimm, *et al.*,¹ in the analysis of the experimental data of Doty and Yang¹⁷ on PBLG in DCA—EDC mixtures. When converted by eq 3, the specific rotation they assigned to perfect helix gives an f_N as small as 0.9.

Evaluation of Transition Parameters

It has been shown in Parts VI³ and VII²⁰ of this series that the helical content f_N of a polypeptide molecule having N degree of polymerization is represented to a good approximation by the equation:

$$f_N = f - 2f^{3/2}(1-f)^{1/2}/N\sqrt{\sigma} \quad (4)$$

provided that $\sqrt{\sigma} \ll 1$, $N \gg 1$, and $N\sqrt{\sigma} > 2$. Here f denotes the value of f_N for infinitely large N and is related to σ and u by a simple equation as shown previously.^{3,20} Equation 4 predicts that a plot of f_N (at fixed solvent conditions) versus N^{-1} gives a straight line whose ordinate intercept I and slope S are equal to f and $-2f^{3/2}(1-f)^{1/2}/\sqrt{\sigma}$, respectively. We have shown that experimental determination of I and S leads to the evaluation of $\sqrt{\sigma}$ and $\ln u$ by the relations:^{3,20}

$$\sqrt{\sigma} = - (2I/S)[I(1-I)]^{1/2} \quad (5)$$

$$\ln u = \frac{-2I(2I-1)}{S} \quad (6)$$

The values of $\sqrt{\sigma}$ and $\ln u$ obtained in this manner refer to the external conditions (solvent species or composition and temperature) in which f_N is determined as a function of N . When $\ln u$ is obtained as a function of temperature under a fixed solvent condition, the enthalpy change ΔH accompanying the formation of helix from random coil may be calculated by means of the relation:

$$\Delta H = -R \frac{\partial(\ln u)}{\partial(1/T)} \quad (7)$$

Eq 4 is concerned with a monodisperse sample of polypeptide, but it is still valid for polydisperse samples if N in the equation is replaced by its number average \bar{N}_n .³ It is for this reason that we have determined the number-average molecular weights for the four polypeptide samples chosen.

We now calculate necessary values of f_N for the application of eq 4 from measured values of $[m']_{436}$ by making use of eq 3. Figure 5 illustrates for a DCA—EDC (65 : 35) mixture the resulting plots of f_N vs. \bar{N}_n^{-1} as a function of temperature. In agreement with the theoretical prediction, the data points at each fixed

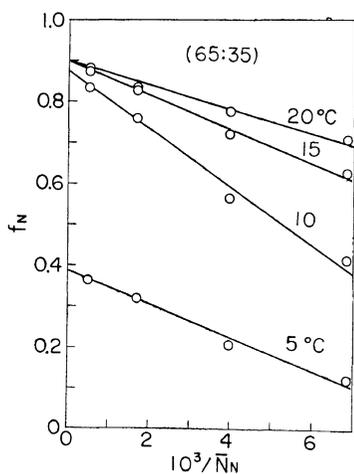


Figure 5. Plots of f_N vs. \bar{N}_N^{-1} for PBLG in a DCA—EDC (65:35) mixture.

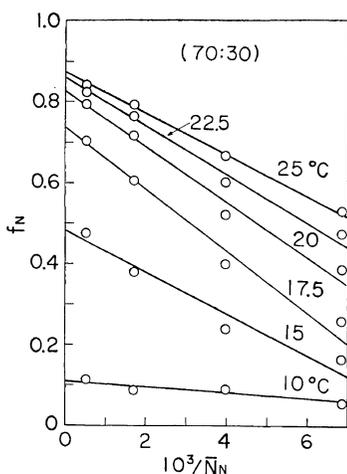


Figure 6. Plots of f_N vs. \bar{N}_N^{-1} for PBLG in a DCA—EDC (70:30) mixture.

temperature follow a straight line over the range of abscissa indicated. Figures 6 and 7 show similar results for other DCA—EDC mixtures. One can observe that the plotted points at relatively high temperatures (as shown in Figure 7) either scatter considerably or even manifest a trend to follow a curve convex downward, so that no sufficient accuracy may be claimed of the straight lines (hence their I and S) drawn through such points. The scatter of the plotted points is chiefly attributed to the difficulty of handling the mixed solvents. It was found that

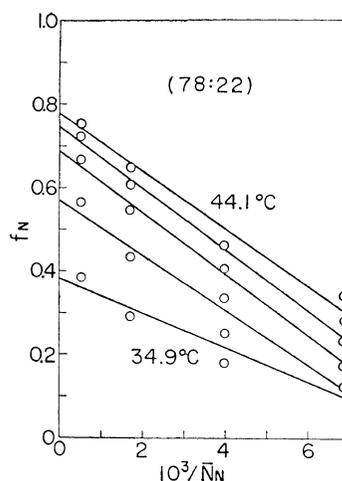


Figure 7. Plots of f_N vs. \bar{N}_N^{-1} for PBLG in a DCA—EDC (78:22) mixture at 44.1, 41.7, 39.2, 37.1, and 34.9°C from top to bottom.

a small variation in DCA content or in the quality of DCA itself affected the observed values of $[m']_{436}$ and hence the transition curve. In all cases treated, the slope S first increased with f , passed through a maximum, and finally decreased as f approached unity. This behavior is the same as that observed with poly- N^5 -(3-hydroxypropyl)-L-glutamine in mixtures of methanol and water by Okita, *et al.*³

The numerical results derived from Figures 5, 6, and 7 are summarized in Table II. The seventh column of the table lists the transition temperature T_c defined as the temperature at which $\ln u$ vanishes. It is seen that the values of $\sqrt{\sigma^-}$ at fixed solvent composition stay nearly constant in the temperature region where the substantial portion of the helix—coil transition occurs and that they show no systematic variation with solvent composition. Thus in the range of the solvent composition studied we have $(0.95 \pm 0.13) \times 10^{-2}$ as an average value of $\sqrt{\sigma^-}$ for the system of PBLG and DCA—EDC mixtures. At temperatures well above T_c , where the helical content approaches a limiting value on the helix side, $\sqrt{\sigma^-}$ appears to increase markedly with temperature. However, we are not confident of whether this trend is real or not.

Figure 8 shows plots of $\ln u$ vs. $1/T - 1/T_c$ for the three DCA—EDC mixtures studied; the data are shown only for the range of T in which $\sqrt{\sigma^-}$

Helix—Coil Transition of Poly(γ -Benzyl-L-glutamate)**Table II.** Numerical values of the transition parameters σ , $\ln u$, and ΔH for PBLG in DCA—EDC mixtures

Solvent composition (volume ratio of DCA to EDC at 25°C)	Temp, °C	f	$\sqrt{\sigma} \times 10^2$	$\ln u \times 10^2$	ΔH at T_c , cal/mol	T_c , °C
65 : 35	5	0.387	0.93	-0.43	800 \pm 50	5.1
	10	0.874	0.82	1.84		
	15	0.898	1.3 ₁	3.44		
	20	0.899	(1.83)	4.85		
70 : 30	5	0.03 ₀	—	-5.2 ₄ ^a	780 \pm 50	15.2
	10	0.108	1.00	-2.53		
	15	0.482	0.93	-0.07		
	17.5	0.736	0.85	0.91		
	20	0.826	0.92	1.57		
	22.5	0.860	0.99	2.06		
	25	0.873	1.13	2.54		
78 : 22	34.9	0.382	0.90	-0.44	580 \pm 60	36.3
	37.1	0.573	0.85	0.25		
	39.2	0.691	0.86	0.72		
	41.7	0.746	0.88	1.00		
	44.1	0.778	0.94	1.26		
average ^b			0.95 \pm 0.13			

^a Calculated by eq 5 and 6 with $\sqrt{\sigma}$ assumed to be 0.95×10^{-2} .

^b Averaged over all values of $\sqrt{\sigma}$ obtained; 0.13 being the standard deviation.

Table III. Comparison of reported values of σ and ΔH for PBLG in various DCA—EDC mixtures

Solvent composition (volume ratio of DCA to EDC at 25°C)	ΔH , cal/mol	$\sqrt{\sigma} \times 10^2$	Polypeptide concn, % (wt/vol)	Source
75 : 25	525 \pm 80	0.56 \pm 0.11	3	Karasz and O'Reilly ⁴
79 : 21	950 \pm 20	0.99	0	Ackermann and Neumann ⁵
82 : 18		1.13		
76 : 24 and 65 : 35	890 \pm 130	1.4 \pm 0.2		Zimm, Doty, and Iso ¹
	750 \pm 100			Kagemoto and Fujishiro ⁷
	650 \pm 30			Giacometti, <i>et al.</i> ⁶
65 : 35	800 \pm 50	0.95 \pm 0.13	0.7~1.0	This Work
70 : 30	780 \pm 50		0.5~0.8	
78 : 22	580 \pm 60		0.5	

has been found to stay nearly constant. It is seen that the data points for different solvent compositions follow curves having different

downward curvature. Literally taken, this result implies that the enthalpy change ΔH for the helix—coil transition of PBLG in the

solvent considered decreases with increasing temperature and the extent is more conspicuous for higher DCA content. The values of ΔH at $T = T_c$ obtained according to eq 7 are given on the sixth column in Table II, and are plotted against T_c in the inset of Figure 8.

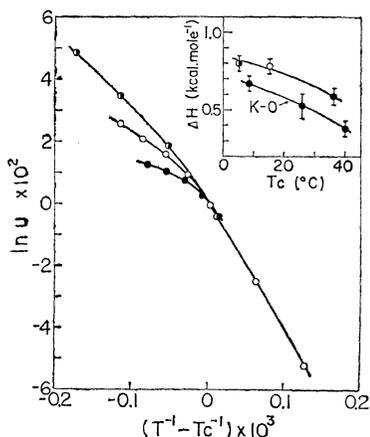


Figure 8. Plots of $\ln u$ vs. $(1/T - 1/T_c)$ for three DCA-EDC mixtures: ●, (65 : 35), ○, (70 : 30), and ●, (78 : 22). The inset illustrates the dependence of ΔH on T_c . K-O indicates the data of Karasz and O'Reilly.⁴

Comparison with Reported Values

We are prepared to compare the values of $\sqrt{\sigma}$ and ΔH from the present study with those reported by previous authors⁴⁻⁷ who evaluated ΔH by direct calorimetric measurements. Table III shows such a comparison, where the data by Zimm, *et al.*,¹ who used a curve-fitting method, are indicated for the sake of reference. The fourth column indicates the range of polypeptide concentration in which these values were determined. This kind of information is important for a meaningful comparison to be made if, as Ackermann and Neumann⁵ have shown, both ΔH and $\sqrt{\sigma}$, especially the former, depend appreciably on polymer concentration. Taking our values not to be much removed from those at infinite dilution, it is seen that, as far as $\sqrt{\sigma}$ is concerned, they are in good agreement with the values at infinite dilution deduced by Ackermann and Neumann.⁵ However, both of them are larger than the values reported by Karasz

and O'Reilly,⁴ who obtained them at as high polymer concentrations as about 3%. On the other hand, our values of ΔH lie between those of Karasz and O'Reilly and of Ackerman and Neumann, but compare well with the results obtained from heat-of-dilution measurements by Giacometti, *et al.*,⁶ and by Kagemoto and Fujishiro.⁷

Essentially, the method we used in the present work to evaluate $\sqrt{\sigma}$ and ΔH is a kind of curve-fitting method, but it seems superior to the method of Zimm, *et al.*,¹ in that it involves no trial-and-error process and can be adapted to the case where these parameters vary with temperature. For a system with temperature-dependent $\sqrt{\sigma}$ and ΔH there will be obtained different values for these parameters depending on what portion of an experimentally given curve of f_N vs. temperature is considered important in fitting theory to experiment. Part of the difference between our values and those of Zimm, *et al.*, observed in Table III may be accounted for by this point; in curvefitting we placed under special consideration the portion of transition curves where f_N changed from about 0.1 to 0.9, whereas Zimm, *et al.*, had attempted it over a nearly complete range of transition with a single set of temperature-independent values of $\sqrt{\sigma}$ and ΔH .

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