Sequential Polypeptides. 2. Helix–Coil Transition in Poly(γ-benzyl ε-carbobenzoxy-L-lysyl-L-glutamate)

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ABSTRACT: Helix–coil transitions of an alternating copolypeptide poly(γ-benzyl ε-carbobenzoxy-L-lysyl-L-glutamate) (poly[Lys(Z)-Glu(OBzl)]) in binary mixtures of dichloroacetic acid (DCA) and ethylene dichloride (EDC) were investigated by optical rotatory dispersion (ORD). The ORD data obtained were analyzed to evaluate the equilibrium constant s for helix formation and the cooperativity parameter σ. The values of $\sigma^{1/2}$ are substatially independent of temperature and solvent composition, yielding 0.4×10^{-2} on the average. The transition enthalpy ΔH_c at the transition point decreases from 291 to 154 cal mol^{-1} as the DCA content is increased from 56 to 64 vol %. At about 20 °C and at 58 vol % DCA, poly[Lys(Z)-Glu(OBzl)] in binary mixtures of DCA and EDC undergoes a solvent-induced transition. This DCA content is intermediate between those for poly(γ-benzyl L-glutamate) and poly(ε-carbobenzoxy-L-lysine) in the same binary solvent and close to that of an equimolar random copolymer of the two peptides. However, the s of poly[Lys(Z)-Glu(OBzl)] is not a geometrical mean of those of the parent homopolypeptides. This fact suggests that the helical conformation of the copolypeptide is stabilized by a conformational induction operative between adjacent residues.

Many synthetic polypeptides are known to assume an α-helical conformation in appropriate solvent conditions, but the stability of their helix under a given solvent condition differs for different polypeptides.[2–5] Typical examples are poly(γ-benzyl L-glutamate) (PBLG) and poly(ε-carbobenzoxy-L-lysine) (PCBL) in *m*-cresol; the former is essentially helical,[6,7] while the latter undergoes a conformational transition from random coil to helix at about 25 °C with raising temperature.[8–11] Since these polypeptides have the same backbone structure, the two peptide residues, when incorporated in a single copolypeptide molecule, may be involved in a single helix sequence, provided that the solvent condition is appropriate. The question then is how the stability of the α helix of a copolypeptide is related to those of the constituent homopolypeptides. We are particularly concerned with a statistical mechanical interpretation of our experiments in this series of copolypeptide studies.

For a given pair (or set) of peptide residues, three types of copolypeptides are conceivable: random, block, and sequential. Most of the published investigations concerning copolypeptides have aimed at establishing empirical structure–conformation relationship.[2,3,12] Recently Scheraga and collaborators[13,14] have developed a procedure, which they called the "host–guest technique," to analyze helix–coil transition in random copolypeptides. This is based on a statistical theory in which some simplifying assumptions were introduced to take into account the fact that the individual chains in a given copolypeptide sample may have different residue arrangement. No such complication exists in block and sequential polypeptides. It is for this reason that these two types of copolypeptides rather than random copolypeptides have been chosen for our series of investigations. Especially, alternating copolypeptides provide us with one of the simplest systems for investigating the effect of interactions between different types of residues on the helix stability. Then poly(γ-benzyl ε-carbobenzoxy-L-lysyl-L-glutamate) (poly[Lys(Z)Glu(OBzl)]) has been chosen for the present study. Its synthesis and characterization have been reported elsewhere,[15] and the helix–coil transitions of its parent homopolypeptides, PBLG and PCBL, have also been investigated in detail in our previous publications.[5,16,17] The work described here deals with a similar analysis of helix–coil transition curves of poly[Lys(Z)-Glu(OBzl)] in mixtures of dichloroacetic acid (DCA) and ethylene dichloride (EDC) and compares the results with those for PBLG,[5,16] PCBL,[8,17] and their random copolymers.[18,19]

Experimental Section

Polypeptide Samples. From a number of poly[Lys(Z)-Glu(OBzl)] samples synthesized previously,[15] samples having approximately the same intrinsic viscosities were combined and separated into narrow-distribution fractions by fractional precipitation with tetrahydrofuran as solvent and cyclohexane as precipitant. Some of these fractions were further separated into three fractions. Finally, five fractions were chosen from them and subjected to column fractionation to remove low molecular weight components; use was made of a thermostatted column packed with fine glass beads together with tetrahydrofuran–cyclohexane mixtures as eluent. The resulting middle fractions were used for physical measurements after being freeze dried from dioxane solutions. Tetrahydrofuran, cyclohexane, methyl ethyl ketone (MEK), and EDC were fractionally distilled after being dried with appropriate reagents.[20] DCA was purified and polymer solutions in mixtures of EDC and DCA were prepared according to the procedures described previously.[8,21]

Molecular Weight Determinations. Number-average molecular weights $\overline{M}_n$ of five poly[Lys(Z)-Glu(OBzl)] fractions were determined by osmotic pressure measurements with MEK of 25 °C as the solvent. Osmometers of the Zimm–Meyerson type fitted with adequately conditioned Sartorious membranes were used. Osmotic pressures were determined to 0.001 cm of solution height by a travelling microscope with the accuracy of ±1% at the lowest concentration studied. The accuracy was higher than this at higher concentrations. Figure 1 shows the results from the osmotic pressure measurements. The data points for each sample follow closely a straight line, giving the ordinate intercept and slope with reasonable accuracy. The numerical results obtained from these data are summarized in Table I. It is suggested from preliminary light-scattering data that the polydispersity index $\overline{M}_w/\overline{M}_n$ may not exceed 1.5 for all these fractions.

Optical Rotation Measurements. Optical rotatory dispersions (ORD) of five poly[Lys(Z)-Glu(OBzl)] fractions were studied at temperatures from 0 to 70 °C with mixtures of DCA and EDC of different compositions as solvent. The measurements were made in the range of wavelengths from 320 to 520 nm by use of a JASCO Model ORD/UV-5 optical rotatory dispersion recorder, and the recorded ORD curves were analyzed by means of the Moffitt–Yang equation with λ_0 taken to be 212 nm. Solvent compositions and polymer concentrations were determined gravimetrically. In the ensuing discussion, the solvent compositions are expressed by the volume percent of DCA at 25 °C.

Results and Discussion

Determination of Helical Fraction. Figure 2 shows plots of the Moffitt parameter b_0 against temperature for two samples of different molecular weights in mixtures of DCA and EDC of the indicated compositions. In the 48 vol % DCA mixture, b_0 stays almost constant at −520 over the range of temperatures from 0 to 70 °C and for all molecular weights. The copolypeptide undergoes thermal transitions of inverse

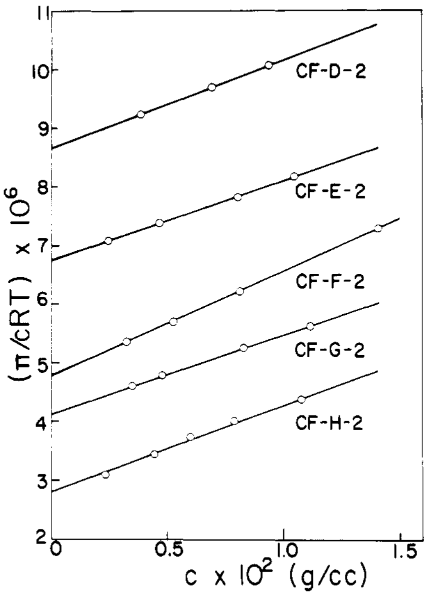

Figure 1. Plots of π/cRT vs. concentration c for poly[Lys(Z)-Glu(OBzl)] in methyl ethyl ketone at 25 °C.

Table I
Results of Osmotic Pressure Measurements on Poly[Lys(Z)-Glu(OBzl)] Fractions in Methyl Ethyl Ketone at 25 °C

Sample code	$\overline{M}_n \times 10^{-4}$	$A_2 \times 10^4$, mol mL g^{-2}
CF-D-2	11.5	1.5
CF-E-2	14.8	1.4
CF-F-2	20.9	1.8
CF-G-2	24.3	1.4
CF-H-2	35.5 ± 0.5	1.4

type in the 58 and 65 vol % DCA mixtures. Theoretically, b_0 should be independent of molecular weight in the random coil conformation as well as in the helical conformation.[5] Thus the data shown in Figure 2 are taken to mean that the copolypeptide is completely α helical in the 48 vol % mixture and characterized by a b_0^H of −520. In the 65 vol % mixture at temperatures below 15 °C, b_0 is +70 for the two molecular weights, and hence b_0^C, the b_0 for random coil, may be taken to be +70. With these values of b_0^H and b_0^C, helical fractions f_N were calculated from b_0 by the relation

$$f_N = (b_0 - b_0^C)/(b_0^H - b_0^C) \tag{1}$$

Determination of Helix-Coil Transition Parameters *s* and *σ*. According to the theories of helix-coil transition in polypeptides,[4,5,22] the helical fraction f_N of a homopolypeptide with an N degree of polymerization is a complicated function of N, the helix-coil equilibrium constant s, and the cooperativity parameter σ. Okita et al.[23] showed that this function can be simplified to give

$$f_N = f - 2f[f(1-f)]^{1/2}/N\sigma^{1/2} \tag{2}$$

provided that the conditions $N \gg 1$, $N\sigma^{1/2} > 2$, and $\sigma^{1/2} \ll 1$ are satisfied. Here f is the value of f_N for infinite N and related to s and σ by

$$f = \tfrac{1}{2}[1 + z/(1+z^2)^{1/2}] \tag{3}$$

with $z = (\ln s)/2\sigma^{1/2}$. Equation 2 predicts a linear dependence of f_N on $1/N$, which, combined with eq 3, provides a means of estimating s and σ from measurements of f_N as a function of N.[5,23] The helix-coil transition enthalpy ΔH can then be calculated from

$$\Delta H = -R\, \partial(\ln s)/\partial(1/T) \tag{4}$$

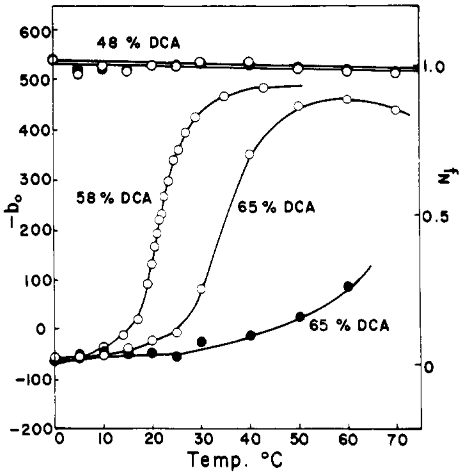

Figure 2. Temperature dependence of the Moffitt parameter b_0 for poly[Lys(Z)-Glu(OBzl)] samples with $\overline{M}_n$ of 35.5 × 10^4 (○) and 11.5 × 10^4 (●) in DCA-EDC mixtures. The solvent composition is indicated by vol % of DCA at 25 °C. The ordinate on the right-hand side gives helical fraction f_N.

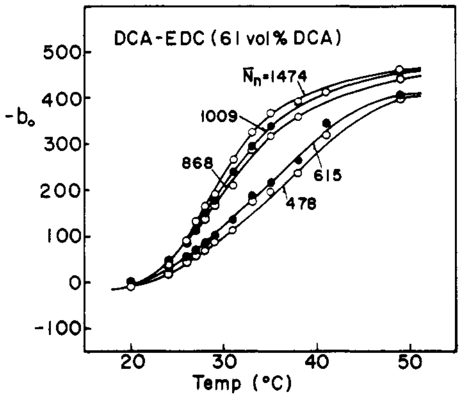

Figure 3. Temperature dependence of the Moffitt parameter b_0 in a DCA-EDC mixture (61 vol % DCA) as a function of number-average degree of polymerization $\overline{N}_n$.

In the analysis to follow, we assume that the same theoretical formulation applies to alternating copolypeptides.

Data in DCA-EDC Mixtures. It was found in the previous study[15] that poly[Lys(Z)-Glu(OBzl)] as well as its parent homopolypeptides, PBLG and PCBL, underwent thermal transitions of inverse type in mixtures of DCA and EDC. This has been confirmed by the data in Figure 2 and similar data given below. Figure 3 shows plots of b_0 vs. temperature for five fractions of different molecular weights in a DCA-EDC mixture (61 vol % DCA). Poly[Lys(Z)-Glu(OBzl)] displays a typical example of inverse thermal transitions which depend significantly on molecular weight. All these b_0 values were converted to f_N by using eq 1 to construct the plots of f_N vs. $1/\overline{N}_n$ shown in Figure 4, where $\overline{N}_n$ is the number-average degree of polymerization. The data points at each temperature are fitted to a straight line as predicted by eq 2, and the s and σ at that temperature can be determined with a fair degree of accuracy. Table II gives a summary of the numerical values of the transition parameters in the 61 vol % DCA mixture. Similar transition curves were obtained for four DCA-EDC mixtures of compositions between 56 and 64 vol % DCA and analyzed in the same way. Some typical values for the transition parameters are shown in Table III to illustrate how they depend on solvent composition at fixed temperatures. It is seen that the values of $\sigma^{1/2}$ in the substantial portion of the transition region are independent of temperature and solvent composition within the accuracy of their determination,

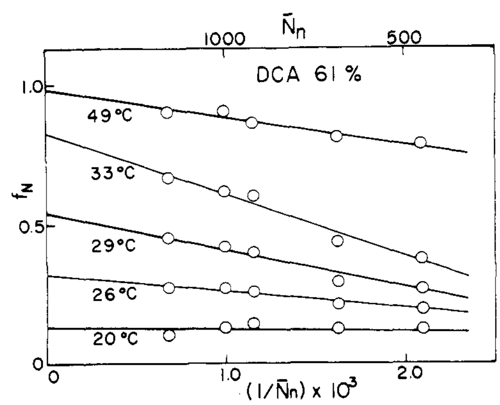

Figure 4. Plots of f_N vs. $1/\bar{N}_n$ for poly[Lys(Z)-Glu(OBzl)] in a DCA–EDC mixture (61 vol % DCA) at indicated temperatures. The ordinate on the top gives $\bar{N}_n$. The solid lines represent the theoretical values calculated for the respective values of f and $\sigma^{1/2}$ given in Table II.

Table II
Numerical Values of the Transition Parameters for Poly[Lys(Z)-Glu(OBzl)] in a DCA–EDC Mixture (61 vol % DCA)

Temp, °C	f	$\sigma^{1/2} \times 10^2$	$(\ln s) \times 10^2$
20	0.134	1.30_4	-2.80_3
24	0.247	0.47_3	-0.55_5
26	0.316	0.47_4	-0.37_5
27	0.405	0.44_3	-0.17_2
28	0.478	0.39_5	-0.03_5
29	0.548	0.40_1	0.07_7
31	0.704	0.33_2	0.29_7
33	0.800	0.32_2	0.48_3
35	0.855	0.32_3	0.65_0
38	0.900	0.32_7	0.87_1
41	0.930	0.35_8	1.20_6
49	0.964	0.43_4	2.11_3

Table III
Typical Values for the Transition Parameters in DCA–EDC Mixtures at 29 °C

Solvent composition, DCA vol %	f	$\sigma^{1/2} \times 10^2$	$(\ln s) \times 10^2$
56	0.944	0.51_1	1.97_2
58	0.943	0.33_0	1.26_0
61	0.548	0.40_1	0.07_7
62.6	0.186	0.29_7	-0.48_0
64	0.123	0.32_3	-0.74_2

yielding an average value of 0.4×10^{-2}. Figure 5 shows plots of ln s vs. $1/T$ for poly[Lys(Z)-Glu(OBzl)] in the five DCA–EDC mixtures examined. The data points for each solvent mixture appear to follow a straight line except in the case of the 62.2 vol % mixture, for which the curve is concave downward. Figure 6 shows plots of b_0 vs. temperature for the highest molecular weight fraction CF-H-2 in DCA–EDC mixtures of the indicated DCA contents. As the DCA content is decreased the transition shifts to lower temperature and becomes sharper. Table IV lists the transition temperature T_c (defined as the temperature at which ln s vanishes), the cooperativity parameter σ, the transition enthalpy at T_c, ΔH_c, and $\Delta H_c/\sigma^{1/2}$ in the five DCA–EDC mixtures studies. The quantity $\Delta H_c/\sigma^{1/2}$ is a measure of the sharpness of the transition, according to the Applequist equation:[24]

$$\partial f/\partial(1/T) = -\Delta H_c/4R\sigma^{1/2} \qquad (\text{at } T = T_c) \qquad (5)$$

Table IV shows that this measure decreases with increasing DCA content, which restates the fact noted above that the transition becomes more diffuse with increasing DCA content.

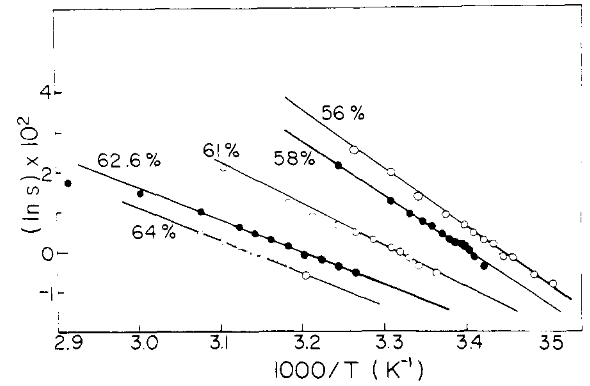

Figure 5. Plots of ln s vs. $1/T$ for poly[Lys(Z)-Glu(OBzl)] in DCA–EDC mixtures of indicated DCA compositions.

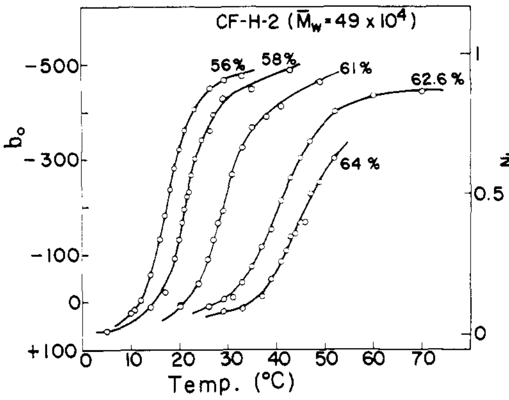

Figure 6. Plots of b_0 vs. temperature for the highest molecular weight fraction CF-H-2 in DCA–EDC mixtures of the indicated DCA compositions.

Table IV
Numerical Values of the Transition Parameters for Poly[Lys(Z)-Glu(OBzl)] in DCA–EDC Mixtures

Solvent composition, vol % DCA	T_c, °C	$\sigma^{1/2} \times 10^2$	ΔH_c, cal mol^{-1}	$\Delta H_c/\sigma^{1/2}$, Kcal mol^{-1}
56	17.3	0.42_1	291	69.1
58	20.8	0.39_4	271	68.8
61	28.6	0.39_0	198	50.8
62.6	39.7	0.41_7	162	38.8
64	45.8	0.41_9	154	36.8

In Table V are compared the DCA contents and the transition parameters for PBLG,[16] PCBL,[17] a BLG–CBL random copolymer (45.7 mol % BLG),[18] and poly[Lys(Z)-Glu(OBzl)], for which the transition temperatures were located near 20 °C. The DCA content for poly[Lys(Z)-Glu(OBzl)] is intermediate between those for PBLG and PCBL and close to that of the random copolymer. Figure 7 shows plots of T_c vs. DCA content for PBLG, PCBL, the BLG–CBL random copolymer, and poly[Lys(Z)-Glu(OBzl)] in DCA–EDC mixtures. The plot for the alternating copolymer appears midway between those of PBLG and PCBL and is located closely left to that for the random copolymer. It should be noted that the sharpness of the transition, when estimated in terms of $\Delta H_c/\sigma^{1/2}$, is approximately the same for PBLG, PCBL, and poly[Lys(Z)-Glu(OBzl)] but that it is significantly lower for the random copolymer. The behavior of the random copolymer appears to conform to the theoretical prediction by von Dreele et al.,[13]

Table V
The Transition Parameters for PBLG, PCBL, a BLG–CBL Random Copolymer, and Poly[Lys(Z)-Glu(OBzl)]

Sample	Solvent composition, vol % DCA	T_c, °C	$\sigma^{1/2} \times 10^2$	ΔH_c, cal mol^{-1}	$\Delta H_c/\sigma^{1/2}$, Kcal mol^{-1}
PBLG[16]	72	23.5	0.93	720	77.4
Poly[Lys(Z)-Glu(OBzl)]	58	20.8	0.39	271	68.8
PCBL[17]	34	20.5	0.32	292	91.3
Random copolymer[a]	61.5	22			25
Poly[Lys(Z)-Glu(OBzl)] calcd[b]	58	46		244	

[a] Random copolymer of γ-benzyl L-glutamate and ϵ-carbobenzoxy-L-lysine (45.7 mol % BLG) taken from Nakajima and Hayashi.[18] [b] Calculated on the basis of eq 7 and with the use of the homopolymer data (see text).

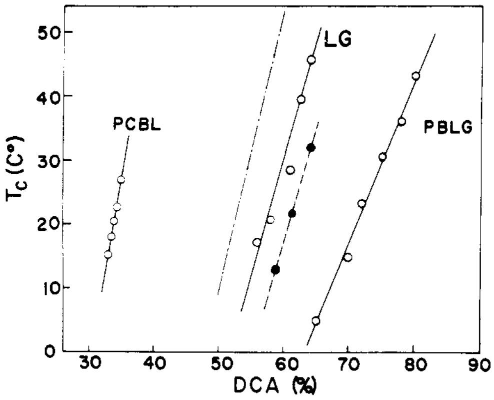

Figure 7. T_c against DCA compositions for PBLG, PCBL, BLG–CBL random copolymer (broken curve), poly[Lys(Z)-Glu(OBzl)] (indicated by LG), and an alternating copolypeptide in which the arithmetic mean of the free-energy differences of the corresponding homopolypeptides (dot-dash curve) is assumed to calculate s.

Table VI
Comparison of the Extrapolated and Calculated s_L in a Mixture of DCA and EDC (61 vol % DCA)

Temp, °C	s_L Extrapolated[a]	s_L Calcd[b]
20	0.768	0.858
24	0.781	0.888
26	0.788	0.887
27	0.791	0.889
28	0.794	0.890
29	0.797	0.890
31	0.803	0.891
33	0.808	0.891
35	0.813	0.892
38	0.820	0.893
41	0.827	0.896
49	0.842	0.909

[a] Calculated by eq 8 with the homopolymer data.[5,17] [b] Calculated by eq 7 from the copolymer data (see text).

but the data are not detailed enough to permit a further analysis.

Theoretical Analysis. The results presented above clearly indicate that the transition of an alternating copolymer is closely related to those of its parent homopolypeptides. In order to elucidate a possible molecular mechanism for this correlation, we have attempted extending the homopolymer theories to alternating copolymers.

Theoretically, s is related to the difference in free energy, ΔG, between the helical and random coil residues in a polypeptide molecule by

$$s = \exp(-\Delta G/RT) \qquad (6)$$

A reasonable first approximation to the free-energy difference ΔG for an alternating copolypeptide is to assume that it is the arithmetic mean of the free-energy differences of the corresponding homopolypeptides. One may then write for poly[Lys(Z)-Glu(OBzl)]

$$s = \exp[-(\Delta G_G + \Delta G_L)/2RT] = (s_G s_L)^{1/2} \qquad (7)$$

where the subscripts G and L refer to BLG and CBL residues, respectively. The values for s_G and s_L in DCA–EDC mixtures are available as functions of temperature over certain ranges of DCA content.[5,16,17] They are expressed by the form[5,16,17]

$$s(T,x_D) = s_0(T)/[1 + K(T)x_D] \qquad (\text{for } s_G \text{ and } s_L) \quad (8)$$

where $s_0(T)$ and $K(T)$ are the temperature-dependent parameters characteristic of the polypeptide and solvent system. In mixtures of DCA and EDC, all the DCA molecules are assumed to exist in the form of cyclic dimer and x_D represents the mole fraction of DCA dimer. Substitution of these s_G and s_L into eq 7 gives the s for the alternating copolypeptide as a function of T and x_D. The condition of $s = 1$ combined with eq 7 should give the transition temperature T_c as a function of x_D for the copolypeptide.

The last row of Table V lists the numerical results calculated in this way for the 58 vol % DCA mixture. The predicted ΔH_c value is in fair agreement with the observed one, but the agreement is not satisfactory for T_c. A similar disagreement can be seen in Figure 7, in which the predicted T_c–DCA content relation (dot-dash line) is compared with the experimental data for poly[Lys(Z)-Glu(OBzl)]. Further calculations showed that no T_c exists for DCA contents above 61 vol %, which obviously contradicts the actual observations. One may thus conclude that eq 7 with the reported s_G and s_L cannot be a satisfactory representation of the transition behavior of poly[Lys(Z)-Glu(OBzl)] in DCA–EDC mixtures. One might ascribe this failure of eq 7 to the errors in s_L, since the s_L values employed in the calculation were estimated by a long-range extrapolation of the values obtained in the range 33–35 vol % DCA to the range 56–64 vol % DCA. However, this may not be the case, because examination of the original data for PBLG and PCBL indicates that the errors in s_L associated with the extrapolation are within ±4% and that the s_G is more accurate. It must be also noted that an attempt at analyzing the copolymer data in Figure 5 by means of eq 8 proved unsuccessful.

Next we assume that the form of eq 7 remains correct but either of s_G and s_L or both of them take different values from those for the respective homopolypeptides. This is the case in which conformational induction occurs between adjacent BLG and CBL residues. On the assumption that s_G is not affected by the presence of CBL residues, the values of s_L leading to the best agreement between the calculated and observed s of the copolypeptide were sought by trial and error. Table VI compares the resulting s_L values with the values obtained by the extrapolation explained above. It can be seen that at any temperature examined the former value is larger than the latter by a factor which is beyond the errors in extrapolated s_L. Literally taken, this means that the ΔG for an adjacent pair of BLG and CBL residues is lower than the sum of those for BLG and CBL residues. This conclusion is consistent with a recent finding by Nishioka[25,26] that the helix stability of BLG–CBL–BLG block copolymers in the same solvent system can be explained if a certain number of CBL residues located on the block boundaries are given a value of s_L larger than that of the other CBL residues. In contrast, in their theory of random copolypeptides, Scheraga and collaborators[13,14] assumed that the statistical weight matrix associated with a given residue would be independent of the kind of its nearest neighbor residues. This assumption is equivalent to the approximation basic to eq 7 that the free-energy differences are additive. As far as the BLG–CBL pair in DCA–EDC mixtures is concerned, such an assumption is not likely to hold due to the conformational induction. However, it may be premature to conclude this to be a general trend, because this effect may vary with different pairs of residues and also with different arrangement of residues. We are now engaged in its study, with a series of sequential polypeptides consisting of BLG and CBL residues.

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Vibrational Analysis of Peptides, Polypeptides, and Proteins. 3. α-Poly(L-alanine) †

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ABSTRACT: Starting with a force field transferred from our earlier studies on β-polypeptides, we have calculated the optically active normal vibration frequencies of α-helical poly(L-alanine) and poly(L-alanine-*N-d*). The 47/13 helical structure was used, and all atoms were included. Only small modifications to the force field were required, and most of these could be justified. The analysis indicates that amide II′ is in Fermi resonance with one component of CH_3 asymmetric bend, thus leading to a small modification of C–N and C=O stretching force constants. The agreement between calculated and observed Raman and infrared bands is quite good. This has encouraged us to calculate the influence of small structural changes on the spectrum as a means of explaining the observed effects of temperature changes.

In the two previous papers in this series[1,2] we have developed a detailed force field for β-polypeptides and applied this force field to the analysis of the antiparallel chain-rippled sheet structure of poly(glycine I) [$(\text{GlyI})_n$] and to the antiparallel chain-pleated sheet structures of poly(L-alanine) [$(\text{Ala})_n$] and poly(L-alanylglycine) [$(\text{Ala-Gly})_n$]. In order that vibrational spectroscopy be a useful tool in studying the conformation of peptide systems, it is important to know how this force field can be extended to structures other than the β sheets. We have, therefore, undertaken the analysis of the vibrational spectrum of α-helical $(\text{Ala})_n$. In studying the effects of deuteration on the IR spectrum and of temperature on the IR and Raman spectra, we have also tried to understand the influence of intramolecular forces on helical stability.

The structure of α-$(\text{Ala})_n$ has been studied by x-ray diffraction by many workers[3–6] and is known to be a right-handed α helix with 47 residues in 13 turns. These helices are hexagonally close packed in the crystal, the chain sense being random.[4]

There is an extensive literature on IR and Raman studies of α-$(\text{Ala})_n$.[7–18] Almost all of these studies were carried out at room temperature, whereas our spectra were also obtained at low as well as high temperatures. Recently[17,19] Raman spectra of N-deuterated species have been reported. We have also obtained such spectra, as well as IR spectra, in the 1600–100-cm^{-1} region.

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† Dedicated to the 80th Birthday of Maurice L. Huggins