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Conformational Induction in Block Copolypeptide of γ-Benzyl L-Glutamate and ε-Carbobenzoxy L-Lysine

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ABSTRACT: Conformations of triblock copolypeptide $[Glu(OBzl)]_m$—$[Lys(Cbz)]_n$—$[Glu(OBzl)]_m$ in *m*-cresol and in mixtures of dichloroacetic acid (DCA) and 1, 2-dichloroethane (EDC) were studied by optical rotatory dispersion measurements. It was found that the polypeptide was essentially helical in *m*-cresol, while it underwent a conformational transition from random coil to helix with raising temperature in mixtures of DCA and EDC of appropriate compositions. In order to elucidate the correlation between these transition behaviors of the copolypeptide and those of the constituent homopolypeptides, the Zimm–Bragg–Nagai formalism of helix-coil transition in homopolypeptide has been extended to triblock copolypeptides. The helical fraction is expressed as a function of the transition parameters s and σ of the two residues and the degree of polymerization of each block. The helix formation in *m*-cresol can be reasonably interpreted by this theory if s and σ are given experimental values of the corresponding homopolypeptides. In mixtures of DCA and EDC, however, the observed helical fraction was always larger than the theoretical value computed by using the homopolypeptide parameters. The copolymer data and the homopolymer data can be explained consistently by this theory if the helical conformation is assumed to be stabilized at each of the block boundaries.

KEY WORDS Block Copolypeptide / γ-Benzyl L-Glutamate / ε-Carbobenzoxy L-Lysine / Helix-Coil Transition / Helix Stability / Conformational Induction / Zimm–Bragg Parameter / Optical Rotatory Dispersion / Statistical Mechanical Theory /

It was shown in a previous publication[1] that poly(ε-carbobenzoxy L-lysine) ($[Lys(Cbz)]_n$) flanked with blocks of poly(γ-benzyl L-glutamate) ($[Glu(OBzl)]_n$) is essentially α-helical, in spite of the fact that an isolated $[Lys(Cbz)]_n$ chain of similar length is completely randomly coiled.[2–5] This typically illustrates the effect of conformational induction in copolypeptide that has been observed with various combinations of peptide residues.[6–11] The extent of conformational induction is expected to vary with the solvent condition under which the polymer is studied as well as with the residue combination. It seemed, therefore, interesting to examine the conformation of triblock copolypeptide $[Glu(OBzl)]_m$—$[Lys(Cbz)]_n$—$[Glu(OBzl)]_p$ in solvents of varying helix-supporting power. In the work described below, mixtures of dichloroacetic acid (DCA) and 1, 2-dichloroethane (EDC) have been chosen as the solvent, since detailed information is available about the helix-coil transitions of constituent homopolypeptides in these mixtures.[2,11–14]

According to the well-established theories,[11,15,16] the conformation of a helix-forming homopolypeptide in a dilute solution is characterized by three parameters, *i.e.*, the degree of polymerization of the polypeptide N, the equilibrium constant for helix formation s, and the helix-initiation parameter σ. A number of attempts[15,17–26] have been made to extend these theories to copolypeptides, with additional assumptions and approximations being introduced in some cases. A typical example is the treatment of Scheraga and collaborators[17,25–33] of random copolypeptides, which they have called the "host-guest technique." It consists in analyzing helix-coil transition curves

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of random copolypeptides of two kinds of residues, the host and guest residues. One of the most important assumptions in this is that the statistical weight of a given residue is independent of the kinds of its nearest neighbor. This assumption may be justified for combinations of similar residues, and in fact, these authors have provided evidence for this.[27,29] However, there are some cases in which it does not seem to hold.[11] Therefore, its validity must be tested with different combinations and arrangements of residues.

In the present series of studies, we are treating two types of copolypeptides, *i.e.*, the block and sequential; both of these have specified residue arrangements. This paper aims at interpreting the experimental data for helix-coil transitions of block copolypeptides in statistical mechanical terms. The necessary theoretical expressions are derived on the basis of Nagai's theory of helix-coil transition. They are used to predict characteristic features of helix formation in block copolypeptide and are applied to detailed data for helix-coil transitions of $[Glu(OBzl)]_m$—$[Lys(Cbz)]_n$—$[Glu(OBzl)]_p$ in mixtures of DCA and EDC. This triblock copolypeptide exhibited an appreciable conformational transition at room temperature in DCA/EDC mixtures in which $[Glu(OBzl)]_n$ was almost perfectly helical, but $[Lys(Cbz)]_n$ randomly coiled.[1] Therefore, the transition parameters of the homopolypeptides evaluated at the respective compositions must be extrapolated to the solvent composition range in which the conformation of the copolymer is studied. To check the validity of the extrapolation procedure employed, we attempted a direct determination of the transition parameters for $[Glu(OBzl)]_n$ under the necessary solvent conditions. The results are also described below.

EXPERIMENTAL

Five samples of $[Glu(OBzl)]_m$—$[Lys(Cbz)]_n$—$[Glu(OBzl)]_p$ prepared and characterized in a previous study,[1] and six $[Glu(OBzl)]_n$ samples from our stock were subjected to optical rotatory dispersion (ORD) measurements. Their molecular weights and residue compositions are given in Table I. It has been shown that these are reasonably homogeneous with respect to molecular weight and composition.[1] All the triblock copolypeptide samples are actually $[Lys(Cbz)]_n$ flanked with $[Glu(OBzl)]_n$ chains of equal length. Optical rotation and ORD measurements were made according to the procedure employed in our previous studies.[2,12-14]

Table I. Molecular weights of block copolypeptides and $[Glu(OBzl)]_n$ used

Sample code	$\bar{M}_W \times 10^{-4}$	$\bar{N}_W$	$\bar{N}_G$	$\bar{N}_L$
GLG-12	1.31	58.5	51.9	6.6
GLG-22	1.39	61.1	49.6	11.5
GLG-331	1.64	71.0	50.8	20.2
GLG-42	1.79	74.9	40.5	34.4
GLG-52	2.16	88.5	36.4	52.1
A-2	0.476	21.8		
An-4	0.85[a]	39		
A-3	1.06	48.4		
A-43	1.46	66.7		
An-22	3.38	154		
E-2	23.7	1068		

[a] Viscosity-average molecular weight obtained from the intrinsic viscosity in dichloroacetic acid at 25°C.[40]

THEORETICAL

Basic Equations

The conformation of an α-helix-forming polypeptide in solution can be described by the theory of Zimm and Bragg[34] and of subsequent authors.[11,15,35-39] Extention of these theoretical formulation to copolypeptides has been attempted by many authors[15,17-26] to predict various interesting aspects of helix-coil transitions in copolypeptide. However, the theoretical expressions so far presented are not necessarily in the form directly applicable to our present data. Thus, here, we follow Nagai's formulation[39] in order to develop a theory adaptable to block copolypeptides, especially of the type G—L—G.

According to Nagai's theory, the partition function Z_N of an α-helix-forming chain consisting of N residues can be written as,

$$Z_N = \boldsymbol{e}_1 \boldsymbol{P}^{N-2} \boldsymbol{e}_N \tag{1}$$

with the transition probability matrix $\boldsymbol{P}$. The reader should consult pertinent references[11,39] as for the definition of $\boldsymbol{P}$. Since the first and the N-th residues are assumed not to be involved in a helical section, the end vectors associated with these residues are written

$$\boldsymbol{e}_1=(0, 0, 0, 1, 0, 0, 1)$$
$$\boldsymbol{e}_N=(0, 0, 0, 0, 1, 1, 1)^T \quad (2)$$

where the superscript T denotes the transpose of a vector. The matrix $\boldsymbol{P}$ can be expanded in its eigenvalues λ_i ($i=1, 2, 3, 4$) as

$$\boldsymbol{P}=\sum_{i=1}^{4}\lambda_i\boldsymbol{u}(\lambda_i)\boldsymbol{v}(\lambda_i) \quad (3)$$

where $\boldsymbol{u}(\lambda)$ and $\boldsymbol{v}(\lambda)$ are the right-hand and left-hand eigenvectors of $\boldsymbol{P}$ given by

$$\boldsymbol{u}(\lambda)=\left(1, \frac{s\sigma^{1/2}}{\lambda^2}, \frac{s}{\lambda}, \frac{s\sigma^{1/2}}{\lambda^2}, \frac{\lambda-s}{\sigma^{1/2}}, \frac{\lambda-s}{\sigma^{1/2}}, \frac{\lambda-s}{\sigma^{1/2}}\right)^T \quad (4a)$$

$$\boldsymbol{v}(\lambda)=c(\lambda)\left(1, \frac{\sigma^{1/2}}{\lambda^2}, \frac{\lambda-s}{s}, \frac{\lambda-s}{s\sigma^{1/2}}, \frac{\sigma^{1/2}}{\lambda}, \frac{\sigma^{1/2}}{\lambda^2}, \frac{\lambda-s}{s\sigma^{1/2}}\right) \quad (4b)$$

with

$$c(\lambda)=\lambda(\lambda-1)F(\lambda) \quad (5)$$

and

$$F(\lambda)=1/[4\lambda^2-3(1+s)\lambda+2s] \quad (6)$$

Substitution of eq 3 into eq 1 gives

$$Z_N=\sum_{i=1}^{4}\lambda_i{}^{N-2}[\boldsymbol{e}_1\boldsymbol{u}(\lambda_i)\boldsymbol{v}(\lambda_i)\boldsymbol{e}_N]$$
$$=\sum_{i=1}^{4}\lambda_i{}^{N}(\lambda_i-s)F(\lambda_i) \quad (7)$$

Next we derive the partition function for a triblock copolypeptide of the type G—L—G. As in the case of homopolypeptide, it is assumed that the statistical weight of a central residue, in a given set of three consecutive residues, is determined by a joint conformation of the three residues concerned, only this time, the arrangement of the residues has to be taken into account. To a good approximation, each residue, excepting those located around G—L and L—G block boundaries, may give the statistical weights characteristic of the corresponding homopolypeptide. At least four residues at a block boundary need a separate consideration, because they lie in different residue arrangements from each other.

First we shall examine the case in which each residue gives the statistical weight matrix characteristic of the corresponding homopolypeptide irrespective of its location. This is the assumption employed in Scheraga's treatment of random copolypeptides. This assumption implies that the statistical weights of the boundary residues are approximated by the geometric means of the corresponding weights of the two residues L and G. It does not seem to have a serious effect on average quantities of the molecule, since the population of the boundary residues is relatively small in the block copolypeptides treated here. However, its validity has to be tested with experimental data. In cases where it turns out to be inadequate, the theory may be modified by assigning different statistical weights to each of the boundary residues in question.

Let us condiser a triblock copolypeptide G_m—L_n—G_p having m, n, and p residues on each block. Applying Nagai's theory to this polypeptide, with the assumption introduced above, we obtain the partition function Z_N of the form:

$$Z_N=\boldsymbol{e}_1\boldsymbol{P}_G{}^{m-1}\boldsymbol{P}_L{}^{n}\boldsymbol{P}_G{}^{p-1}\boldsymbol{e}_N \quad (8)$$

where the subscripts L and G refer to L and G residues, and $N=m+n+p$. After similar manipulation employed to derive eq 7, we get

$$Z_N=\boldsymbol{e}_1\left[\sum_{i=1}^{4}\lambda_{G_i}{}^{m-1}\boldsymbol{u}(\lambda_{G_i})\boldsymbol{v}(\lambda_{G_i})\right]$$
$$\times\left[\sum_{j=1}^{4}\lambda_{L_j}{}^{n}\boldsymbol{u}(\lambda_{L_j})\boldsymbol{v}(\lambda_{L_j})\right]\left[\sum_{k=1}^{4}\lambda_{G_k}{}^{p-1}\boldsymbol{u}(\lambda_{G_k})\boldsymbol{v}(\lambda_{G_k})\right]\boldsymbol{e}_N$$
$$=\sum_{i,j,k}^{4}\lambda_{G_i}{}^{m-1}\lambda_{L_j}{}^{n}\lambda_{G_k}{}^{p-1}\boldsymbol{v}(\lambda_{G_i})\boldsymbol{u}(\lambda_{L_j})\boldsymbol{v}(\lambda_{L_j})\boldsymbol{u}(\lambda_{G_k})$$
$$\times[\boldsymbol{e}_1\boldsymbol{u}(\lambda_{G_i})\boldsymbol{v}(\lambda_{G_k})\boldsymbol{e}_N]$$
$$=\sum_{i,j,k}^{4}\lambda_{G_i}{}^{m}\lambda_{L_j}{}^{n}\lambda_{G_k}{}^{p}\boldsymbol{v}(\lambda_{G_i})\boldsymbol{u}(\lambda_{L_j})\boldsymbol{v}(\lambda_{L_j})\boldsymbol{u}(\lambda_{G_k})$$
$$\times(\lambda_{G_i}-s_G)F(\lambda_{G_k}) \quad (9)$$

where λ_{G_i} and λ_{L_j} ($i, j=1, 2, 3, 4$) are the roots of the characteristic equation of $\boldsymbol{P}$, with s and σ being replaced by s_G and σ_G and s_L and σ_L, respectively. With Z_N thus derived, the average number of intact hydrogen bonds of the copolypeptide relative to N, θ_N, can be calculated by

$$\theta_N=N^{-1}[\partial(\ln Z_N)/\partial(\ln s_G)+\partial(\ln Z_N)/\partial(\ln s_L)] \quad (10)$$

The analytical expression for θ_N thus obtained is very complicated[40] and hence it is not shown here. A matrix representation of eq 10 was also used for numerical computation.

Theoretical Prediction

It was shown in a previous paper[7] that the central Lys(Cbz) block in a triblock copolypeptide

$[Glu(OBzl)]_m$—$[Lys(Cbz)]_n$—$[Glu(OBzl)]_p$ is forced to take up an α-helical conformation in *m*-cresol, in which $[Lys(Cbz)]_n$ itself undergoes a sharp thermal transition of an inverse type. At first, we shall try to interpret this conformational induction by the copolymer theory developed above. $[Glu(OBzl)]_n$ is known to form a stable α-helix in *m*-cresol at room temperature with the transition parameters of $s_G=1.61$ and $\sigma_G^{1/2}=0.04$,[41] while the thermal transition of $[Lys(Cbz)]_n$ is characterized by very small $\sigma_L^{1/2}$ of 0.0025 and transition temperature around 25°C.[2-5] In the discussion to follow, these parameter values are used to test theoretical predictions on the helix stability of the triblock copolypeptide under the solvent condition considered here.

Figure 1 illustrates the dependence of θ_N on the degree of polymerization N_L of the central *L* block as a function of s_L, with the degree of polymerization N_G of each flanking block being fixed at 20. It is seen that θ_N first increases, passes through a maximum, and then decreases gradually as N_L is increased. However, for $s_L>0.95$, θ_N changes only slightly with N_L at least up to an N_L of 60. These values of θ_N are converted to the average number of intact hydrogen bonds N_h and plotted against N_L in Figure 2. Unless s_L is very small, N_h first increases linearly with N_L. This implies that a short *L* block sandwiched between G blocks is forced to become helical. As N_L is increased further, N_h decreases toward an assymptotic value

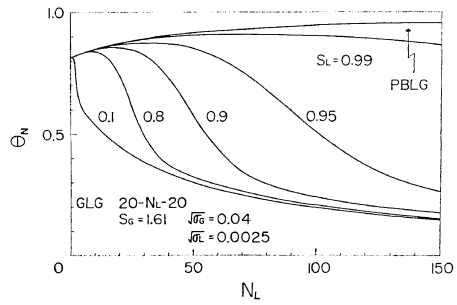

Figure 1. Helical fraction θ_N against degree of polymerization N_L of the central *L* block as a function of s_L, with the degree of polymerization N_G of each flanking *G* block being fixed at 20; 20—N_L—20. The curve labeled PBLG indicates the theoretical values of θ_N for *G* polymer having the same degree of polymerization as the triblock copolypeptide. The parameters chosen are $s_G=1.61$, $\sigma_G^{1/2}=0.04$, and $\sigma_L^{1/2}=0.0025$.

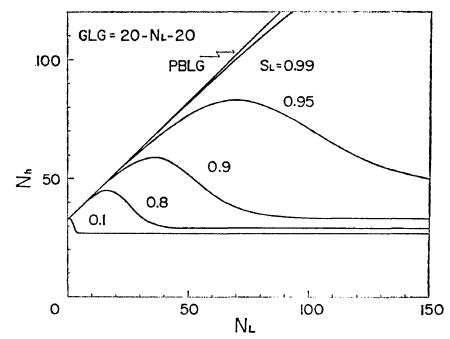

Figure 2. Average number of helical residues N_h against N_L as a function of s_L, with N_G being fixed at 20. The same parameters as those in Figure 1 are used.

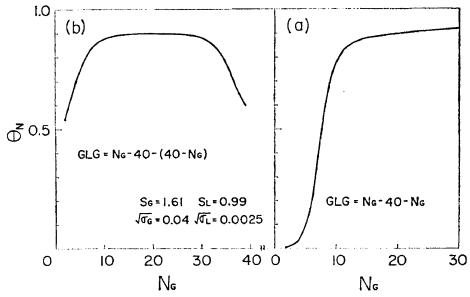

Figure 3. Plots of θ_N *vs.* N_G, with N_L being fixed at 40. The parameters chosen are $s_G=1.61$, $\sigma_G^{1/2}=0.04$, $s_L=0.99$, and $\sigma_L^{1/2}=0.0025$.

depending on s_L. However, for s_L close to unity, N_h increases steadily with N_L within the range of N_L examined.

Panel (a) of Figure 3 shows how θ_N varies with N_G for a fixed N_L of 40 and a set of the transition parameters: $s_G=1.61$, $\sigma_G^{1/2}=0.04$, $s_L=0.99$, and $\sigma_L^{1/2}=0.0025$. It is to be noted that an N_G of 8 is large enough to bring about appreciable conformational induction in the central L block, while an isolated L_{40} is completely randomly coiled under the same condition. Panel (b) of the same figure indicates that the difference in chain length of the flanking blocks has little effect on θ_N if the two blocks are longer than 8 residues. This critical length of G chain may be compared with the previous estimate of the minimum chain length required for the formation of an α-helix in $[Glu(OBzl)]_n$, which is between 6 and 10.[6,7,10,41] These theoretical arguments are consistent with the experimental finding that the conformational induc-

tion by the flanking Glu(OBzl) blocks extends over 60 Lys(Cbz) residues in *m*-cresol, because s_L is estimated to be in the range 1.000 ± 0.004,[2,3,5] and because N_G is larger than 18 for all the samples studied. It was, indeed, found that the θ_N values at 20°C estimated from ORD data were in fair agreement with those calculated for $s_L = 0.95 \sim 0.99$.

Figure 4 shows the dependence of θ_N on s_L as a function of s_G for a triblock copolypeptide G_{20}—L_{40}—G_{20}. The conformational induction is seen to occur abruptly at a certain value of s_L, which is larger for smaller s_G. Even when s_G is smaller than unity, θ_N for larger s_L exceeds 0.5, a value which would be expected if only the L residues were in the helical conformation. This implies that the central L block promotes the helix formation in the flanking blocks when the stability of the former helix exceeds that of the latter. No experimental evidence is as yet available for this prediction.

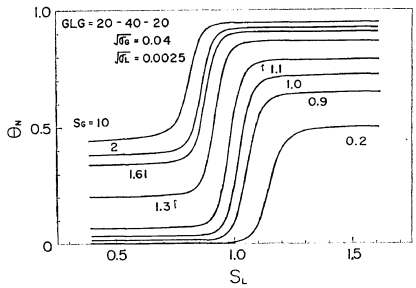

Figure 4. The s_L dependence of θ_N as a function of s_G for a triblock copolypeptide 20—40—20, with $\sigma_G^{1/2} = 0.04$ and $\sigma_L^{1/2} = 0.0025$.

EXPERIMENTAL RESULTS AND DISCUSSION

Figure 5 shows thermal transition curves of sample GLG-331 in DCA/EDC mixtures of different compositions, where the solvent compositions are indicated by a volume ratio of DCA to EDC at 25°C. One can observe that the triblock copolypeptide undergoes a helix-coil transition of inverse type. At fixed temperature, *e.g.*, 25°C, the polymer undergoes a solvent-induced transition between 50- and 55-vol% DCA. This composition range may be compared with 75-vol% for [Glu(OBzl)]$_n$[12,13] and 34-vol% for [Lys(Cbz)]$_n$.[2,14,43,44] It is also interesting to note that an alternating co-

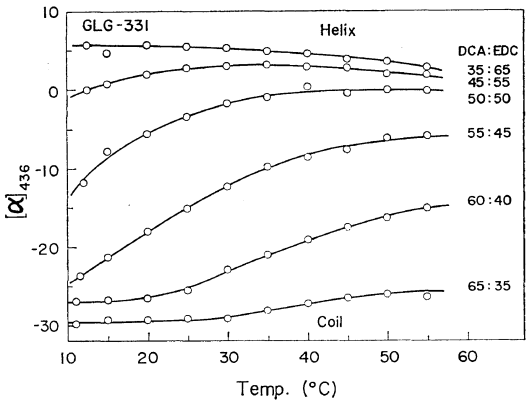

Figure 5. Temperature-dependence of specific rotation at 436 nm, $[\alpha]_{436}$, for sample GLG-331 in DCA/EDC mixtures of different compositions. Compositions are indicated by volume ratios of DCA to EDC at 25°C.

polypeptide [Lys(Cbz)–Glu(OBzl)]$_n$ shows an inverse thermal transition between 53- and 64-vol% DCA.[45,46] When either thermally-induced or solvent-induced, the transition of the triblock copolypeptide proceeds in a single stage. This would not be expected if both the Glu(OBzl) and Lys(Cbz) blocks were to undergo independent transitions.

Figure 6 shows transition curves of [Glu(OBzl)]$_n$ in a DCA/EDC mixture (55-vol% DCA) as functions of molecular weight, where the data for GLG-331 are reproduced for comparison. The transition curve for GLG-331 lies far below that of

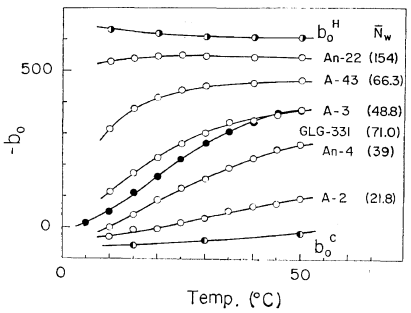

Figure 6. Temperature-dependence of the Moffitt parameter b_0 for [Glu(OBzl)]$_n$ in a DCA/EDC mixture (55-vol% DCA) as a function of weight-average degree of polymerization $\bar{N}_w$. The filled circles represent the data for GLG-331. The b_0^H (◑) and b_0^C (◐) denote b_0 for perfect helix and random coil, respectively, determined for the system DCA/EDC (see text).

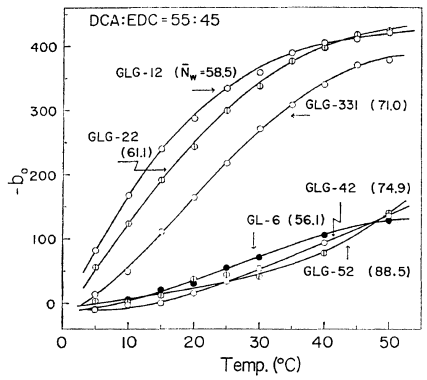

Figure 7. Temperature-dependence of b_0 for block copolypeptides in a DCA/EDC mixture (55-vol% DCA). The filled circles represent the data for GL-6, diblock sample $[Glu(OBzl)]_{30.4}-[Lys(Cbz)]_{25.7}$.[1]

$[Glu(OBzl)]_n$ of similar chain length (A-43) but above that expected for an isolated flanking Glu(OBzl) block ($\bar{N}_G \approx 25$). This implies that the central Lys(Cbz) block partially becomes helical by the interaction of the flanking blocks, just as found in the *m*-cresol solutions. Figure 7 shows the temperature dependence of the Moffitt parameter b_0 for block copolypeptides of different compositions in a DCA/EDC mixture (55-vol% DCA). The transition curve shifts toward higher temperature and becomes gradual as the Lys(Cbz) content is increased. Similar results were obtained with solvent mixtures containing 50- and 60-vol% DCA.

Analysis of $[Glu(OBzl)]_n$ *Data*

Initially the ORD data shown in Figure 6 are analyzed to evaluate the transition parameters s_G and σ_G. As usual, θ_N is calculated from the Moffitt parameter b_0 by

$$\theta_N=(b_0-b_0^C)/(b_0^H-b_0^C) \tag{11}$$

where b_0^H and b_0^C stand for the values of b_0 for perfect helix and random coil, respectively.

The values of b_0^H and b_0^C depend generally on polypeptide and solvent conditions, and have to be determined experimentally. For this purpose, ORD data were obtained using three $[Glu(OBzl)]_n$ samples of different molecular weights (A-2, An-4, and E-2) in DCA/EDC mixtures (20- and 90-vol% DCA). The results are also included in Figure 6. In the 90-vol% mixture, considered to be helix-breaking, b_0 decreases from 63 to 16 as the temperature is raised from 10 to 50°C. On the other hand, in the 20-vol% mixture, which is strongly helix-supporting, b_0 varies from −630 to −610 with the same change in temperature. In either solvent mixture, b_0 hardly changed with molecular weight. Therefore, the b_0 values in these solvent mixtures were taken as b_0^C and b_0^H at the corresponding temperatures. Values of s_G and σ_G were evaluated by fitting the experimental θ_N as function of N to Nagai's theoretical expression.[11,39] A summary of the resulting s_G and σ_G is given in Table II.

Table II. Transition parameters of $[Glu(OBzl)]_n$ in a mixture of 55-vol % DCA at various temperatures

Temp, °C	Observed		$s_{G,extp}$
	s_G	$\sigma_G^{1/2}$	
10	1.11	0.0086	1.13
15	1.13	0.0081	1.15
20	1.14	0.0093	1.16
25	1.15	0.0102	1.17
30	1.16	0.0100	1.18
35	1.17	0.0108	1.18
40	1.17	0.0124	1.19
45	1.17_5	0.0130	1.19
50	1.18_5	0.0130	1.19

In a binary solvent mixture composed of a less polar liquid such as EDC and organic acid such as DCA, the equilibrium constant s depends on both T and the mole fraction of DCA considered as dimer, x_D. It has been shown that[11,13]

$$s=s_0/(1+Kx_D) \tag{12}$$

where s_0 is the value of s in the less polar solvent and K is the association constant between the peptide residue and DCA dimer. K and s_0 are functions only of T and can be expressed as

$$s_0=\exp[-(\Delta H_0-T\Delta S_0)/RT] \tag{13}$$

$$K=\exp[-(\Delta H_a-T\Delta S_a)/RT] \tag{14}$$

where R is the gas constant and ΔH_0, ΔS_0, ΔH_a, and ΔS_a are the parameters independent of T. If the values of these parameters are available, eq 12 combined with eq 13 and 14 permits the estimation of s as a function of T and x_D. Using the reported results for $[Glu(OBzl)]_n$[13] and $[Lys(Cbz)]_n$,[14] we calculated s_G and s_L in a DCA/EDC

mixture (55-vol% DCA) as functions of T (see Table II) and are designated as $s_{G,extp}$ and $s_{L,extp}$, respectively. The values for $[Glu(OBzl)]_n$ thus estimated are in excellent agreement with those directly obtained and listed in Table II, thus justifying the extrapolation procedure based on eq 12. The same procedure was employed to estimate $s_{G,extp}$ and $s_{L,extp}$ in the 50- and 60-vol% DCA mixtures.

Analysis of Copolypeptide Data

Equation 11 may also be used for block copolypeptides, but this time b_0^H and b_0^C must be allowed to vary with the residue composition of the sample. Since b_0 contains the contributions from Glu(OBzl) and Lys(Cbz) residues, it may be written

$$b_0^{\xi}=(b_0^{\xi})_L X+(b_0^{\xi})_G(1-X) \quad (\xi=\text{H or C}) \quad (15)$$

where X denotes the mole fraction of Lys(Cbz) residue and $(b_0^{\xi})_G$ and $(b_0^{\xi})_L$ are the values of b_0 at the specified conformation ξ of Glu(OBzl) and Lys(Cbz), respectively. The reported values of $(b_0^{\xi})_L$[14] and those of $(b_0^{\xi})_G$ obtained above were substituted into eq 15 and 11 to determine the helical fraction of each sample.*

For the triblock copolypeptides treated here, it is assumed that $m=p=N_G/2$ and $n=N_L$. Thus the theory gives θ_N as a function of s_G, σ_G, s_L, σ_L, N_G and N_L. In order to analyze the block copolypeptide data, the values of $\bar{N}_G$ and $\bar{N}_L$ in Table I are used for N_G and N_L. Since σ is essentially independent of solvent composition and temperature,[11-14,16] $\sigma_G^{1/2}$ and $\sigma_L^{1/2}$ are taken to be 0.0095 and 0.003, respectively, the average values reported for these polypeptides in DCA/EDC mixtures.[11,14]

Figure 8 shows transition curves of sample GLG-331 in DCA/EDC mixtures of three different compositions. In this figure, the solid lines represent the theoretical θ_N values calculated by means of eq 10 with the values of $s_{G,extp}$ and $s_{L,extp}$ estimated by eq 12. It is seen that for either solvent mixture, the theoretical values of θ_N are much smaller than the experimental values indicated by circles. Thus, eq 10 combined with the homopolypeptide data is not satisfactory for explaining the transition behavior of the block copolypeptides.

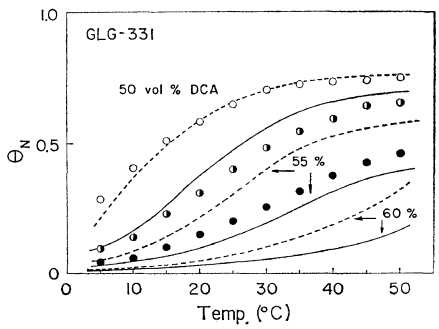

Figure 8. Comparison between theoretical and experimental values of θ_N for sample GLG-331 in three DCA/EDC mixtures. The circles denote expreimental values: ○, 50-vol% DCA; ◑, 55-vol%; ●, 60-vol%. The solid lines represent the theoretical curves calculated without considering the boundary effect, while the dashed lines represent the theoretical curves calculated by taking the boundary effect into account. The values of $s_{G,extp}$ and $s_{L,extp}$ determined by eq 12 and $\sigma_G^{1/2}=0.0095$ and $\sigma_L^{1/2}=0.003$ were used for the calculation.

There are two possible reasons for this failure of eq 10. One of them may be an underestimation of $s_{L,extp}$, which naturally leads to an underestimation of θ_N. The parameters needed to calculate $s_{L,extp}$ were determined by using ORD data taken at low DCA contents. The long-range extrapolation by eq 12 to obtain $s_{L,extp}$ values at DCA contents appropriate to the block copolypeptides studied here may give rise to considerable error in the estimated $s_{L,extp}$. However, examination of the original data for $[Lys(Cbz)]_n$ suggested that such error, if present, should result in an overestimation of $s_{L,extp}$ to some extent. This is contrary to what is expected from the results shown in Figure 8. Thus, the first possibility cannot be of any essential importance.

In an α-helical conformation, one hydrogen bond is to be formed between i-th and $(i+4)$-th peptide residues encompassing the three residues

* Equation 11 with b_0^{ξ} given by eq 15 yields an approximate value of θ_N when $(b_0^H)_G-(b_0^C)_G \neq (b_0^H)_L-(b_0^C)_L$. A more rigorous treatment is to analyze the quantity F_N defined by $F_N=b_0/(b_0^H)_G$, which is theoretically expressed as

$$F_N=(1/N)\sum_{\rho=G,L}\sum_{\xi=H,C} k_{\xi\rho}\langle N_{\xi}\rangle_{\rho}$$

with

$$k_{\xi\rho}=(b_0^{\xi})_{\rho}/(b_0^H)_G$$

where $\langle N_{\xi}\rangle_{\rho}$ denotes the average number of residues ρ in the conformation ξ. It was found that although θ_N and F_N differed numerically, this fact had no serious effect on the result of the analysis.

in between. To be consistent with this structure, the statistical weight matrix $\boldsymbol{P}$ is associated with the central residue in three consecutive residues. In this way, interactions among at least three consecutive residues are taken exactly into theoretical consideration. Thus, it follows that, to the same approximation, four residues at a block boundary should be given statistical weight matrices different from the rest. For simplicity, we assume here that a certain number δN of Lys(Cbz) residues located at the boundary give the statistical weight matrix for Glu(OBzl) residue. The dashed lines in Figure 8 represent the theoretical values calculated with $\delta N=2$. Agreement between theory and experiment is excellent for the 50-vol% mixture, but is not so good for the other mixtures. It has been found that all the experimental data can be brought to moderate agreement with the theoretical values if δN is taken to be 2.5 ± 1.

Next the same assumption of $\delta N=2$ and the $s_{G,extp}$ computed above were used to obtain θ_N as a function of s_L, and the value of s_L which gave the experimental θ_N was sought out by trial and error. The values of s_L thus estimated for different samples at fixed solvent conditions scattered considerably, and their average was denoted by $\langle s_L \rangle$. Figure 9 shows a comparison of the theoretical curves calculated with $\langle s_L \rangle$ thus estimated and the experimental data for three DCA/EDC

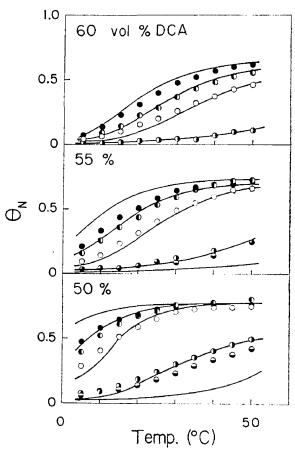

Figure 9. θ_N against temperature for GLG block copolypeptides in three DCA/EDC mixtures. The solid lines represent the theoretical curves calculated by considering the boundary effect into account with $s_{G,extp}$ and $\langle s_L \rangle$: ●, GLG-12; ◐, GLG-22; ○, GLG-331; ◑, GLG-42; ◒, GLG-52.

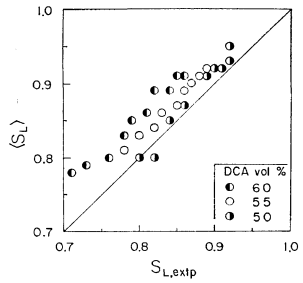

Figure 10. Plots of $\langle s_L \rangle$ against $s_{L,extp}$ as functions of solvent composition: ◑, 50-vol% DCA; ○, 55-vol%; ◐, 60-vol%.

mixtures (50-, 55-, and 60-vol% DCA). The agreement between theory and experiment is satisfactory except for samples GLG-12 and GLG-52. Figure 10 shows plots of $\langle s_L \rangle$ *vs.* $s_{L,extp}$ for the three solvent mixtures. It can be seen that $\langle s_L \rangle$ follows approximately $s_{L,extp}$, which justifies the assumption introduced. Thus we may conclude that the present analysis provides evidence for the conformational induction between adjacent Glu(OBzl) and Lys(Cbz) residues, which helps stabilize the helical conformation. It must be noted, however, that the experimental data are not detailed enough to permit a separate estimate of the two factors involved in this effect, *i.e.*, the number of the boundary residues concerned and their statistical weights. In this connection, it is worthwhile to remark about a recent finding of Itou, *et al.*,[46] that the helix stability of alternating copolypeptide [Lys(Cbz)—Glu(OBzl)]$_n$ in DCA/EDC mixtures is appreciably greater than that expected from the constituent homopolypeptides data. It is assumed in the "host-guest" technique of Scherage that the statistical weight of a given residue is independent of its location. Obviously, this assumption does not hold for the block copolypeptide studied here and the alternating copolypeptide examined by Itou, *et al.*[46] In order to elucidate molecular mechanisms responsible for the conformational induction, investigations into sequential polypeptides of various combinations and arrangements of residues are in progress in our laboratory.

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