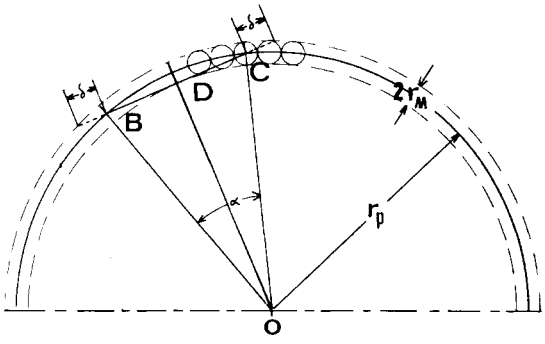

Figure 3. Schematic illustration of the geometry of high electron density particles along the circumference of a low electron density particle within a segregated network ($r_P/r_M \geq 5$).

of one of the immediate M particles, or the error, 2δ, when the particle has originated in the center of some neighboring nth particle not contained in the circumference of interest, will be much smaller than the chord length itself. Consequently, δ will be ignored in the first approximation. By defining the radiu of material M, r_M, equal to 2.5 μm and the radius of material P, r_P, equal to 50 μm, the longest chord that can be inscribed, $\overline{BC}$, equals,

$$\overline{BC} = 2r_P(\sin \tfrac{1}{2}\alpha) \quad \text{(i)}$$

From the Pythagorean theorem,

$$\overline{bO}^2 = \overline{OD}^2 + \overline{BD}^2 \quad \text{(ii)}$$

Since $\overline{BD} = \frac{1}{2}\overline{BC}$, substitution of eq i into eq ii yields,

$$r_P^2 = (r_P - r_M)^2 + [r_P(\sin \tfrac{1}{2}\alpha)]^2 \quad \text{(iii)}$$

Making numerical substitution for r_P and r_M gives $\alpha = 0.635$ rad Solving eq i for chord length, $\overline{BC}$, yields 31 μm.

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(18) For a "segregated" distribution, processing is carried out in such a way that the metallic particles reside only on the original sufaces of the polymeric particles, and the ratio of polymer to metal radii, (r_P/r_M), is large (≥ 5). While from a macroscopic viewpoint a random distribution results for all (r_P/r_M) since the particles used are quite small (r_P or $r_M < 150$ μm), only as lim (r_P/r_M) $\rightarrow$ 1 does a truly "random" distribution result at the microscopic level. This is the important distinction of a "segregated" distribution which, from a practical viewpoint, reduces by sixfold the volume fraction of metal necessary to achieve good electrical conductivity when (r_P/r_M) = 20[6] vs. ca. 1.[7]

Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 11. Lysine Parameters from Random Poly(hydroxybutylglutamine-*co*-L-lysine)[1]

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Received January 26, 1976

ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-lysine with N^5-(4-hydroxybutyl)-L-glutamine, and the thermally induced helix–coil transitions of these copolymers in water, are described. The incorporation of L-lysine was found to decrease the helix content of the polymers at neutral pH. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-lysine) in water were deduced from an analysis of the melting curves in the manner described in earlier papers. The computed values of s indicate that, in the temperature range of 0–60 °C, lysine has a tendency to destabilize helical sequences, this tendency being minimal at ~25 °C and increasing at lower and higher temperatures.

Poly(L-lysine) has been studied extensively both in water and in aqueous salt solutions, and has been shown to exhibit the characteristics of random coil, α helix, or β structure depending on conditions of pH and temperature.[3–9] The value of the standard free energy change, $\Delta G°$, for conversion of an uncharged residue in the random coil state to an uncharged residue at the end of a preexisting α-helical region has been determined previously by application of the theory of Zimm and Rice[10] to the analysis of titration data.[11–13] The temperature dependence of this quantity, in turn, has provided a measure of $\Delta H°$ and $\Delta S°$ for this process. An independent measure of $\Delta H°$ obtained calorimetrically has verified these results.[14] However, it has been assumed in these investigations that the helix-forming ability of an ionizable residue is independent of its state of ionization except for electrostatic repulsions between side chains when the residues are charged. Thus, the Zimm–Bragg helix stability constants,[15] σ and s, obtained from these studies for an uncharged lysine residue

are assumed to reflect the *intrinsic* helix forming property of this residue (either charged or uncharged). It has been demonstrated recently in paper 9 of this series[16] that this assumption is not valid. In that work,[16] it was shown that, under conditions where electrostatic repulsions do not influence the helix–coil transition, there are in fact significant differences between the helix-forming abilities of charged and uncharged glutamic acid residues. These results for glutamic acid were obtained by application of the host–guest technique described and applied in papers 1–10 of this series.[16–25] The present paper concerns the application of this technique to obtain the helix–coil stability constants for a lysine residue at neutral pH. In this procedure, the amino acid whose parameters are sought is incorporated as a minor component into a polyamino acid host whose melting behavior is known. The influence of the guest upon the melting behavior of the host provides a means of determining the intrinsic properties of the guest residue. Specific long-range interactions between guest residues can be eliminated by restricting the composition of guest residues to low values. Thus, as shown previously,[16] the host–guest method can be used to obtain the Zimm–Bragg parameters for charged amino acids.

In the present paper, the preparation of copolymers of L-lysine and N^5-(4-hydroxybutyl)-L-glutamine is described, and the Zimm–Bragg stability constants for a charged lysine residue have been obtained. Synthesis of the water soluble random copolymers of L-lysine with N^5-(4-hydroxybutyl)-L-glutamine (HBG)[26,27] is described in section I. The experimental characterization of these copolymers and their thermal transitions are presented in section II. Finally, in section III, these data are analyzed by means of an appropriate form of the theory[17] to calculate the helix–coil stability parameters for L-lysine in water or 0.1 N KCl at neutral pH. These results are compared with empirical observations on the behavior of this residue in polypeptides and proteins.

I. Experimental Section. Preparation and Characterization of the Copolymers

The synthesis of the copolymers was achieved by first copolymerizing the *N*-carboxyanhydrides (NCA's) of *N*-ε-*tert*-butyloxycarbonyl-L-lysine and γ-benzyl L-glutamate in dioxane using either triethylamine or sodium methoxide as an initiator. The resulting copolymers were then converted to the hydroxybutylglutamine derivatives by treatment with 4-amino-1-butanol. Finally, the *N*-ε-*tert*-butyloxycarbonyl blocking group was removed using 3.5 N HCl to give the final product.

(A) Materials. The solvents used for preparation and recrystallization of the NCA's and for the polymerizations were purified shortly before use. Dioxane was refluxed and then distilled over sodium. Hexane was dried over calcium sulfate and decanted prior to use. Acetonitrile was distilled over P_2O_5. Triethylamine was refluxed and distilled with acetic anhydride, then dried and distilled over KOH. Sodium methoxide was prepared by placing freshly cut sodium in anhydrous methanol and diluting with benzene.[28] Ethyl acetate was dried over Linde Molecular Sieves (4A) and decanted just before use. α-Benzyloxycarbonyl-L-lysine was purchased from Sigma Chemical Co. and *tert*-butyloxycarbonyl azide was purchased from Aldrich. The dichloroacetic acid (DCA) used for the viscosity measurements was obtained from Fisher Scientific Co. and distilled under reduced pressure. Absolute ethanol from Commercial Solvents Corp. and analytical reagent grade anhydrous methanol, ether, and benzene from Mallinckrodt Chemical Works were used without further purification.

(B) Synthesis. *N*-Carboxyanhydrides. α-Benzyloxycarbonyl-L-lysine was reacted with *tert*-butyloxycarbonyl azide according to the procedure reported by Ali et al.[29,30] to give α-benzyloxycarbonyl-*N*-ε-*tert*-butyloxycarbonyl-L-lysine. This product was then subjected to catalytic hydrogenation following standard procedures[31] to obtain *N*-ε-*tert*-butyloxycarbonyl-L-lysine. *N*-ε-*tert*-Butyloxycarbonyl-L-lysine *N*-carboxyanhydride was obtained by the action of phosgene on a suspension of the amino acid (with protected ε-amino group) in the presence of AgCN according to the procedure of Hirschmann et al.[32] Two recrystallizations from ethyl acetate and hexanes gave a 51% yield of colorless material: mp 136–137 °C (lit. mp 123 °C);[32] $[\alpha]^{23}D$ −39.9° (*c* 1.2, CH_2Cl_2) [lit. $[\alpha]^{23}D$ −34.7° (*c* 1.1, CH_2Cl_2)]. Anal. Calcd for $C_{12}H_{20}Nn2O_5$: C, 52.9; H, 7.40; N, 10.3. Found: C, 53.2; H, 7.27; N, 10.0.

γ-Benzyl L-glutamate *N*-carboxyanhydride was also prepared according to the general method described by Hirschmann et al.[32]

Poly[γ-benzyl L-glutamate-*co*-*N*-ε-*tert*-butyloxycarbonyl-L-lysine], Poly[Glu(OBzl),Lys(Boc)], Copolymers I–V. Random copolymers of γ-benzyl L-glutamate and 5 to 20 mol % of *N*-ε-*tert*-butyloxycarbonyl-L-lysine were prepared by polymerizing mixtures of the two *N*-carboxyanhydrides in dioxane with either triethylamine or sodium methoxide as initiator. For polymers III and V, triethylamine initiator was added to give an A/I ratio of 25 and the polymerization reaction was carried out exactly as described in earlier papers of this series.[16] The copolymers I, II, and IV were prepared using sodium methoxide initiation with A/I ratios of 40, 50, and 60, respectively. The progress of these reactions was monitored by assaying for unreacted *N*-carboxyanhydrides using an assay procedure described in paper 10.[25] The reactions were found to be more than 98% complete in a few hours. In both cases the viscous mixtures were slowly poured into 400 ml of vigorously stirred absolute ethanol (for III and V) or acetonitrile (for I, II, and IV). The white fibrous precipitate was collected on a sintered glass filter, washed with ethanol (or acetonitrile), and dried to constant weight over P_2O_5 in vacuo. Yields were generally 81–94%. The chain lengths of these polymers (determined roughly by the relationship of Fujita et al.[33] from the viscosities in dichloroacetic acid) are given in Table I.

Table I
Compositions and Chain Lengths of Unfractionated Poly[Glu(OBzl),Lys(Boc)]

Polymer No.	L-Lysine content of reaction mixture, mol %	L-Lysine content found, mol %	Av mol wt[a] × 10^3	$\overline{DP}$
I	5	4.9%	195	886
II	7.5	7.1%[b]	240	1086
III	10	8.9%	260	1171
IV	10	9.3%	286	1288
V	20	20.5%	236	1053

[a] By viscometry using the relation of Fujita et al.[33] for poly[Glu(OBzl)] in dichloroacetic acid. [b] This value may be in error by as much as 15% because the precautions mentioned in section IE[35,36] had not been taken in the analysis of this sample. However, none of this material was available for reanalysis. Nevertheless, since fraction VIIB of Table II, which was derived from sample II of Table I, was reanalyzed, this error does *not* carry over to the data of Table II.

Poly[N^5-(4-hydroxybutyl)-L-glutamine-*co*-L-lysine], Poly[HBG,Lys], Copolymers VI–X. The copolymers I–V were treated with 4-amino-1-butanol to convert them to the corresponding water-soluble copolymers VI–X according to the previously described procedure.[20] The aminolysis reaction was monitored by assaying for unexchanged γ-benzyl ester groups as described in paper 10.[25] When more than 99% of the benzyl ester groups had been exchanged (6–9 days) the reaction was terminated by pouring the mixture slowly into 100 ml of cold aqueous HCl sufficiently concentrated to give ~3.5 N HCl following neutralization of excess 4-amino-1-butanol in the reaction mixture. The solution was stirred 30 min to ensure complete removal of the *tert*-butyloxycarbonyl protecting group, then diluted to give ~1 N HCl and stirred an additional 2 h. The solution was then dialyzed against H_2O until amines could no longer be detected in the dialyzate by a ninhydrin test.[34]

(C) Removal of *tert*-Butyloxycarbonyl Protecting Group. The Fourier transform ^1H NMR of a 5% solution of sample VIC was measured on a Brüker 90 MHz NMR spectrometer and compared with the spectrum of a model compound, obtained in a similar way. The absence of a resonance corresponding to the *tert*-butyl group indicated that less than 1.0% of the lysyl side chains remained in the blocked state.

(D) Fractionation. The water-soluble copolymers VI–X were fractionated by precipitation in a methanol and ether solvent system. This procedure, described in paper 2,[18] produced from four to eight fractions of each copolymer. Each was dissolved in H_2O, lyophilized, and dried in vacuo.

(E) Determination of Composition. The amino acid compositions of all copolymer fractions were determined on a Technicon amino acid analyzer. The samples were hydrolyzed in 12 N HCl at 105 °C for 48 h in degassed, sealed ampules. In order to eliminate artifact formation which can occur during removal of hydrochloric acid from protein hydrolyzates,[35] the samples were neutralized with sodium hydroxide according to the procedure of Spitz.[36] Analysis of synthetic mixtures of lysine and glutamic acid standards subjected to identical conditions showed that no correction for the destruction of amino acids during hydrolysis was necessary. For samples in which the Glu/Lys ratio exceeded 10 the amino acid ratio was determined relative to an internal standard of Norleucine added to the hydrolyzate. The average experimental error in determination of composition was found to be ±5% for copolymers having a Glu/Lys ratio ≤10, and ~±8% for a Glu/Lys ratio exceeding 10.

(F) Determination of Concentration. The concentration of all copolymer solutions was determined by the Micro-Kjeldahl analysis for nitrogen as described previously.[19] Multiple aliquots of each solution were analyzed. The error in this measurement was found to be ±4%.

(G) Viscometry. The intrinsic viscosity of each of the copolymers I–V was determined in DCA at 25.0 ± 0.1 °C in a Cannon-Ubbelohde semimicro dilution viscometer. Using poly(γ-benzyl L-glutamate) as a model and the relationship determined by Fujita et al.,[33] a rough estimate of the molecular weight was obtained for these polymers.

(H) Optical Purity. The monomeric starting material as well as the acid hydrolyzates of the final copolymers were assayed for the presence of D isomers using the dipeptide method of Manning and Moore.[37] Glutamic acid, lysine, and 4-amino-1-butanol were isolated from the hydrolyzate mixture prior to derivatization. The separation was achieved by means of two scaled-down ion-exchange columns. First a column (60 mm × 5 mm, contained in a disposable pipette) of Dowex AG1-X4 (200–400 mesh) in the hydroxyl form was used to separate the 4-amino-1-butanol from the two amino acids by eluting with water followed by 0.5 N acetic acid. The amino acids were eluted directly onto a second column of Dowex AG1-X4 in the acetate form. Separation was achieved by eluting with 0.5 N acetic acid at a flow rate of 15 ml/h.

The glutamic acid from this separation was coupled with L-Leu-NCA and the resulting diastereoisomeric dipeptides L-Leu-L-Glu and L-Leu-D-Glu were separated and quantitatively determined on a Technicon amino acid analyzer using a sodium citrate elution buffer of pH 3.9 (0.2 N NaCl). The lysine from the above separation was coupled with L-glutamic acid *N*-carboxyanhydrides, and was likewise analyzed for the diastereoisomeric dipeptides. The analysis was accomplished on a Technicon analyzer eluting with a sodium citrate buffer of pH 4.3 (0.2 N NaCl). Appropriate dipeptide standards were prepared from D,L-lysine and D,L-glutamic acid.

(I) Determination of Molecular Weights. The molecular weights of fractions from samples VI–X were determined by the conventional sedimentation equilibrium method using a Beckman Model E analytical ultracentrifuge. All measurements were made in water at pH 11.8 in order to eliminate polyelectrolytic effects arising from the ionization of the ε-amino group of the lysine residues. Viscosity and molecular weight measurements at 25 °C and pH 11.8 both indicated that slow degradation takes place under these conditions. To obtain accurate measurement of the molecular weight, the sedimentation equilibrium measurements were carried out at reduced temperature (5 °C) where this instability was not observed. The solutions were prepared by dialyzing a stock solution (~0.3% w/v) against a large volume of solvent at pH 11.8 and 5 °C. The dialyzate was used as the solvent in the molecular weight measurements, and all dilutions were made using this dialyzate. Following dialysis, the solutions were placed in vials and sealed with serum caps in order to prevent absorption of CO_2 into the alkaline solution. $\bar{M}_w^{app}$ was determined as described in earlier papers for each sample at several concentrations (from 0.1 to 0.3%), and $\bar{M}_w$ was found by extrapolation of these values to zero concentration. $\bar{M}_z$ was computed from the data corresponding to the lowest concentration for each fraction. The accuracy of the molecular weight determinations is ±5%. The partial specific volumes ($\bar{v}$) of the copolymers required for the molecular weight calculation were determined from the amino acid content as described by Cohn and Edsall.[38] A value of $\bar{v}$ = 0.816 for HBG residues was used in the calculation of $\bar{v}$ for the copolymers.[18]

(J) Optical Rotatory Dispersion and Circular Dichroism Measurements. The optical rotatory dispersion (ORD) and the circular dichroism (CD) measurements were made with a Cary Model 60 spectropolarimeter equipped with a Model 6001 CD attachment, as described earlier.[20] Temperature control was maintained to within ±0.2 °C with water-jacketed quartz cells. All solutions were made up fresh prior to use and filtered through 0.45 μ Millipore filters. The helix content (θ_h) is defined as $-b_0/750$, where b_0 is the slope of the Moffitt–Yang plot obtained as described previously.[18] The experimental error in θ_h results from: (a) the error in the concentration (±4%), (b) the error in the values of b_0 assigned to the complete helix and the complete coil (±3%), and (c) the error in the slope of the Moffitt–Yang plot (±2%).

Table II
Characterization of the Fractionated Copolymers

Fraction[a]	Wt of fraction, mg	L-Lys content, mol %	$\bar{M}_w \times 10^{-3}$ [b]	$\bar{M}_z/\bar{M}_w$	$\overline{DP}_w$
VIA	45				
VIB	84				
VIC	188	4.7	128.1	1.06	645
VID	84				
VIE	110				
VIF	52	5.1	72.9	1.11	370
VIIA	30				
VIIB	75	5.5[c]	39.3	1.15	200
VIIC	30				
VIIIA	8				
VIIIB	96	8.3	126.7	1.17	640
VIIIC	84	8.4			
VIIID	82	8.5	45.3	1.22	230
IXA	128				
IXB	159	9.7	161.3	1.07	820
IXC	133				
IXD	89	9.8			
IXE	131	9.8			
XA	88	19.6			
XB	131	20.1			
XC	149	19.5	68.7	1.22	356
XD	68				
XE	77	20.1			
XF	40	19.8	36.7	1.07	190

[a] Samples VI–X are derived from the unfractionated copolymers I–V, respectively. The letters correspond to the fractions obtained in the fractionation procedure. [b] This value was obtained by conventional sedimentation equilibrium (with extrapolation to zero concentration). [c] See footnote *b* of Table I.

II. Results

(A) Characterization of the Copolymers. The composition and the average degree of polymerization ($\overline{DP}_w$) for the unfractionated poly[Glu(OBzl),Lys(Boc)] copolymers are summarized in Table I. Table II summarizes the molecular weight and composition data for the corresponding deprotected, fractionated copolymers poly[HBG,Lys]. The usual decrease in $\overline{DP}_w$ attributed to transaminolysis upon conversion of these polymers to their hydroxyalkylglutamine derivatives is apparent from a comparison of the two tables. However, the fact that the average composition of the fractionated copolymers agrees with that determined for the unfractionated copolymers (with the qualification in footnote *c* of Table II) indicates that no large departures from random copolymerization occurred. In any case it has been amply demonstrated theoretically in paper I[17] of this series that small deviations from randomness do not influence the melting behavior of these copolymers; i.e., as shown in Figure 4 of ref 17, the accumulation of the two *amino acid* components into blocks of fairly large size (for a *given overall* composition of the copolymer) does not affect the melting behavior. The theory of ref 17 has been verified experimentally in ref 18.

Table II provides information about the molecular weight and homogeneity of those fractions used in the theoretical analysis in section III. The apparent molecular weights were found to be concentration dependent, and extrapolation to

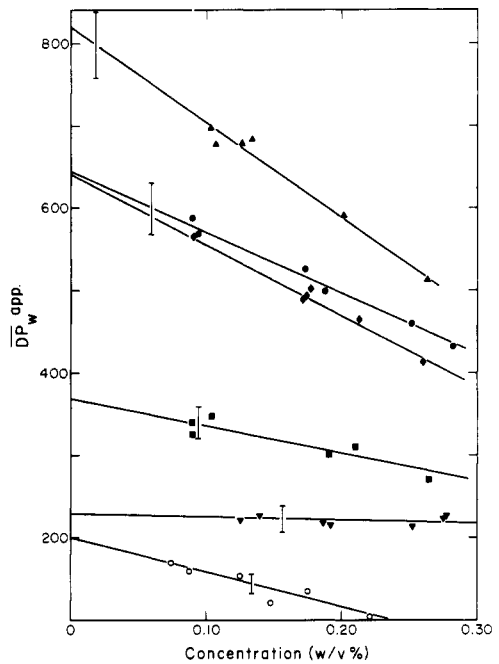

Figure 1. Concentration dependence of molecular weights for fractions used in analysis to obtain σ and s: (▲) 9.7% Lys, $\overline{DP}_w$ = 820 (fraction IXB); (●) 4.7% Lys, $\overline{DP}_w$ = 645 (fraction VIC); (♦) 8.3% Lys, $\overline{DP}_w$ = 640 (fraction VIIIB); (■) 5.1% Lys, $\overline{DP}_w$ = 370 (fraction VIF); (▼) 8.5% Lys, $\overline{DP}_w$ = 230 (fraction VIIID); (0) 5.5% Lys, $\overline{DP}_w$ = 200 (fraction VIIB).

infinite dilution was required to obtain the values of $\overline{DP}_w$. An observed increase in apparent molecular weight with decreasing concentration is generally attributed to nonideality. Although there is roughly a threefold variation in concentration from the meniscus to the bottom of the cell in any equilibrium experiment, there is no evidence of the concentration dependence of molecular weight within any one given run; i.e., the plot of ln c vs. r^2 is linear for each experiment, whereas nonideality would be expected to produce curvature toward the r^2 axis. Such curvature, however, could have been compensated by a curvature in the opposite direction arising from polydispersity of the copolymer fractions, leading fortuitously to a linear ln c vs. r^2 plot. This compensatory effect can explain the fact (shown in Figure 1) that the copolymer fraction with the smallest observable nonideal behavior corresponds to the most heterogeneous fraction ($\bar{M}_z/\bar{M}_w$ = 1.22). The nonideal effect is diminished at increased temperature (25 °C) but, as stated above, the copolymers undergo slow degradation under these conditions of temperature and pH. At 5 °C, however, no degradation was detected over a period of 144 h. The concentration dependence of $\bar{M}_w^{app}$ was not affected by increasing the pH from 11.8 to 12.5, and therefore cannot be attributed to the presence of ionized groups.

The $\bar{M}_z/\bar{M}_w$ ratios given in Table II do not depart significantly from unity. The values obtained indicate that the fractionation procedure yielded relatively homogeneous material.

In the Manning–Moore assay for optical purity the degree of racemization was found to be less than 1 mol % for both the lysine and the HBG residues in representative fractions of the copolymers. All samples were therefore judged to be optically pure.

(B) b_0 for Complete Helix and Complete Coil. For the homopolymer host poly(HBG) studied in paper 2,[18] the value of b_0 corresponding to 100% helix was assigned as −750, and that corresponding to the complete coil was taken as zero. Because the side chain influences the optical rotatory properties of a polypeptide, it was necessary to determine the respective values of b_0 for the copolymers with lysine. Hence, b_0 of the most helical copolymer fraction VIC was determined in trifluoroethanol (a helix-promoting solvent) at 1 °C; and b_0 for fraction XF (19.8% Lys) was measured in DCA at 25 °C. After correcting the data for the dispersion of the refractive index of the solvent, b_0 values of −733 and +3.2 were obtained for 100% helix and coil, respectively. These results demonstrate that the assignments of −750 for complete helix, and zero for complete coil are reasonable ones for analyzing the copolymers. In order to account for uncertainties in these values, we include an error of ±3% in the error analysis of $\theta_{h,exptl}$.

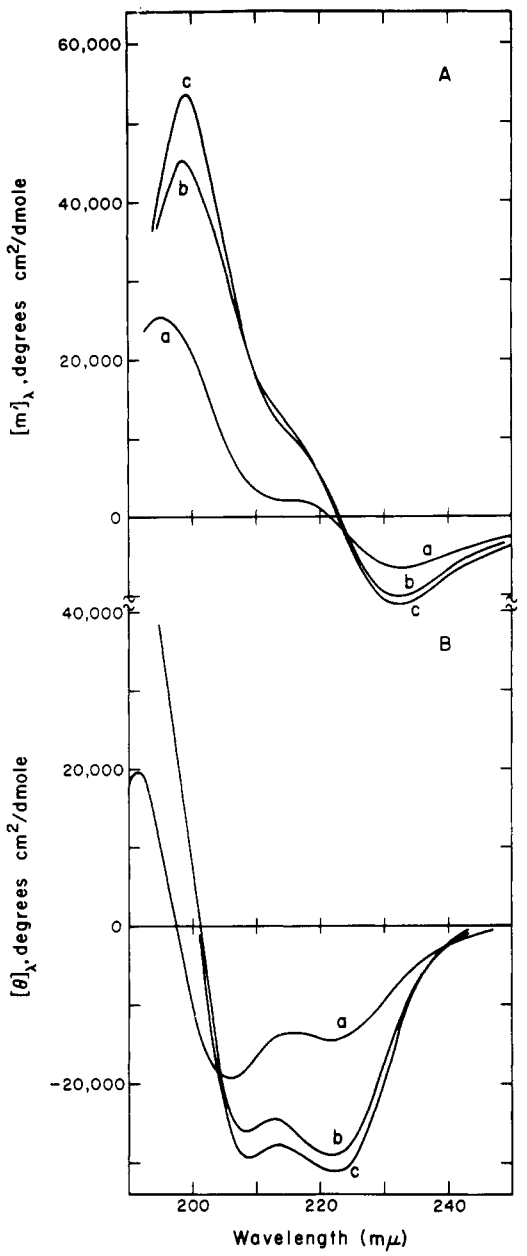

Figure 2. (A) ORD and (B) CD spectra in water at 25 °C and at neutral pH for three poly(HBG,Lys) copolymers: (a) 19.5% Lys, $\overline{DP}_w$ = 356 (fraction XC); (b) 8.3% Lys, $\overline{DP}_w$ = 640 (fraction VIIIB); (c) 4.7% Lys, $\overline{DP}_w$ = 645 (fraction VIC).

(C) ORD and CD Data. The ORD and CD spectra on three poly[HBG,Lys] copolymer fractions representing three different composition ranges are shown in Figure 2. These measurements were carried out in water at neutral pH and at 25 °C. Both the CD and the ORD spectra are interpretable in terms of contributions from right-handed α helix and random coil, the relative contributions from each conformation being

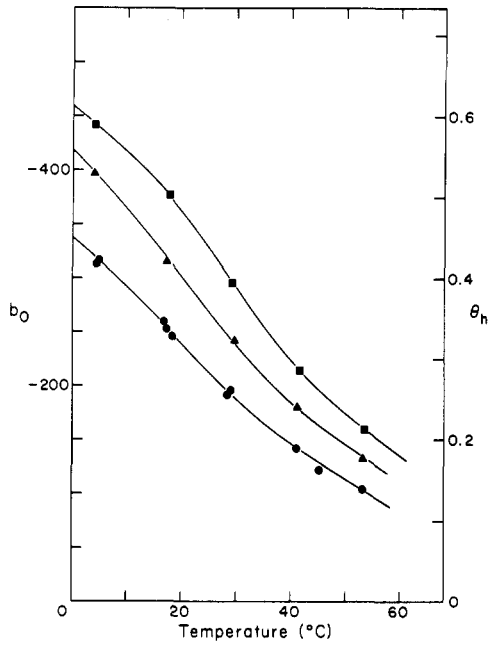

Figure 3. Thermally induced helix–coil transition for copolymer XC (19.5% Lys, $\overline{DP}_w$ = 356): (●) in water; (▲) in 0.1 N KCl; (■) in 0.5 N KCl.

a function of both temperature and copolymer composition.[39,40] Contributions to these spectra arising from β structure are not evident. Similarly, at pH 11.8 where the lysine residues are uncharged, the CD and ORD spectra (not shown here) represent a mixture of right-handed α helix and random coil. The helix content observed at this pH is increased relative to that obtained at neutral pH and, again, there is no evidence of β structure. The spectra obtained at pH 11.8 were reliable only for temperatures less than 10 °C because the copolymers were found to be unstable at higher temperatures.

With increasing temperature, the ORD spectrum in water at neutral pH (not shown here) becomes characteristic of the random coil mixed with small amounts of α helix, indicating that these copolymers undergo a thermally induced transition from α helix to random coil. The thermally induced helix–coil transition curves were obtained by measuring the temperature dependence of b_0 and taking θ_h (fraction helix) to be $-b_0/750$. Transition curves were obtained for all eight of the fully characterized fractions (listed in Table II) in water at neutral pH, in 0.1 N KCl at neutral pH, and in water at pH 11.8. All melting curves obtained at alkaline pH were found to be irreversible because of the instability of these compounds at high pH and elevated temperatures. Because of this irreversibility, no attempt was made to obtain σ and s for lysine in the uncharged state from an analysis of these melting curves. At neutral pH, the helix content of the two characterized fractions of highest lysine composition (19.5 and 19.8% Lys) at any given temperature was found to depend on the concentration of added salt. This behavior is shown for one of these (19.5% Lys, fraction XC) in Figure 3. The helix content was found to increase as the concentration of added salt was increased from 0 to 0.5 N KCl. It is apparent from these data that the conformation of the copolymers of high lysine content is influenced by long-range electrostatic repulsions between the charged lysine side chains. These interactions are partially suppressed in the presence of increasing amounts of salt which acts to shield the charges from one another. The presence of long-range repulsions in these two fractions render them unsuitable for use in the analysis to obtain σ and s since the theories treat only near-neighbor interactions. For the remaining six fractions analyzed, all of which contain less than 10.0% lysine, no differences could be detected between the melting curves obtained in water at neutral pH and those obtained in 0.1 N KCl solutions. Thus, for these copolymers of *low* lysine content, the charged lysine side chains appear to be sufficiently separated from one another so that the helix–coil transition is not influenced by electrostatic repulsions. Therefore, the analysis for σ and s was confined to the six fractions of low lysine content and, since the melting behavior in 0.1 N KCl was indistinguishable from that observed in water, the parameters obtained are equally applicable for describing the behavior of lysine residues under either of these conditions. The melting curves used in the analysis are shown in Figure 4. Each transition was found to be completely reversible, reproducible, and independent of concentration. Two observations can be made from an examination of these transition curves. First, for a given lysine content [cf. 8.3% Lys, $\overline{DP}_w$ = 640, Figure 4A (△); and 8.5% Lys, $\overline{DP}_w$ = 230, Figure 4B (○)], θ_h increases with increasing chain length as is expected. Second, for a given chain length ($\overline{DP}_w \sim 640$, Figure 4A), θ_h is found to decrease with increasing lysine content, thus indicating qualitatively that lysine residues behave as helix breakers. The error symbols shown in these plots arise from uncertainties in the determination of concentration, from error in the slope of the Moffitt–Yang plot, and from the possible error in the choice of b_0 for 100% helix and coil.

III. Discussion

(A) Helix–Coil Parameters for Poly(L-lysine). The melting curves described in section II were analyzed according to the LAPS (Lifson–Allegra–Poland–Scheraga) hierarchy

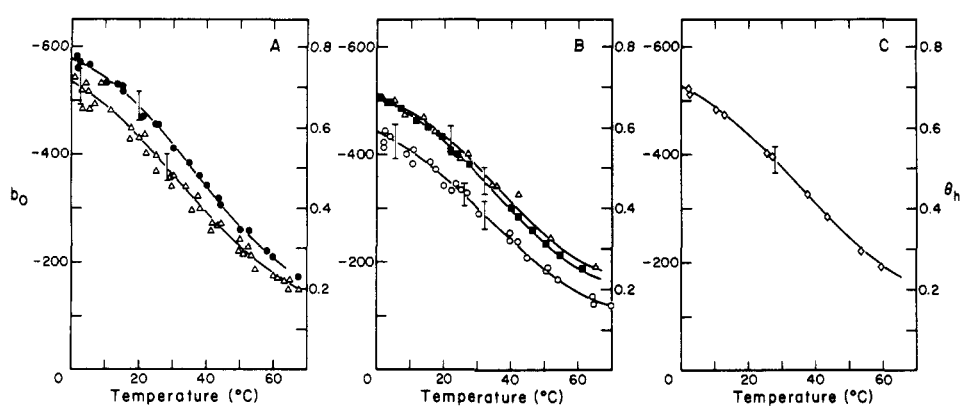

Figure 4. Thermal transitions for L-lysine copolymers in water: (A) (●) 4.7% Lys, $\overline{DP}_w$ = 645 (fraction VIC); (△) 8.3% Lys, $\overline{DP}_w$ = 640 (fraction VIIIB); (B) (△) 5.5% Lys, $\overline{DP}_w$ = 200 (fraction VIIB); (■) 9.7% Lys, $\overline{DP}_w$ = 820 (fraction IXB); (○) 8.5% Lys, $\overline{DP}_w$ = 230 (fraction VIIID); (C) (◇) 5.1% Lys, $\overline{DP}_w$ = 370 (fraction VIF). The points are experimental ones, and the lines represent smoothed experimental curves. The error symbols reflect the experimental errors affecting θ_h.

Table III
Comparison of the Values of θ_h Calculated with Approximate and Exact Theories for Finite Chains[a]

L-Lys content, mol %	$\overline{DP}_w$	Temp, °C	$(\theta_h)_{exptl}$	$(\theta_h)_{theor}$ Lifson[b]	Allegra[c]	Lehman–McTague[c]
8.5	230	0	0.591	0.623	0.624	0.619
		30	0.401	0.438	0.438	0.449
		60	0.195	0.223	0.223	0.223
5.1	370	0	0.700	0.707	0.707	0.694
		30	0.497	0.491	0.492	0.493
		60	0.253	0.255	0.255	0.251
8.3	640	0	0.715	0.695	0.695	0.692
		30	0.483	0.496	0.497	0.504
		60	0.236	0.249	0.248	0.249

[a] The parameters used for hydroxybutylglutamine were taken from Table II of paper 2.[18] [b] The parameters used for L-Lys were obtained by fitting the experimental data with the Lifson theory, as shown in Table IV. [c] The parameters used for L-lysine were obtained by fitting the experimental data with the Allegra theory, as shown in Table IV.

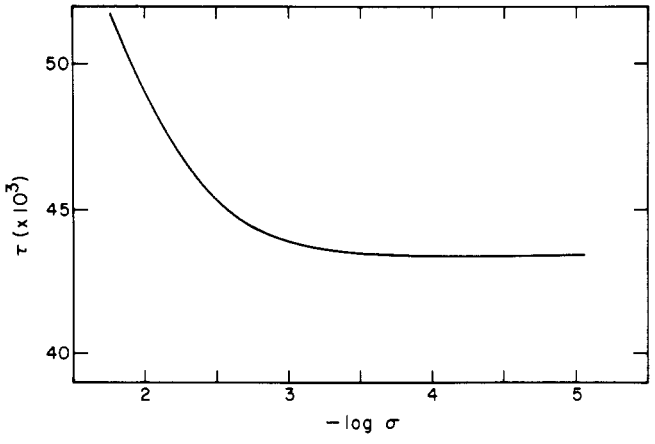

Figure 5. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the lysine copolymers using the Allegra theory.

of approximations to obtain σ and s for poly(L-lysine) in water (or 0.1 N KCl). This procedure has been discussed extensively in previous papers in this series.[17-19] To conserve computer time, the first approximation, corresponding to the theory of Lifson,[41] was used to obtain an initial estimate of σ and s; then these values were refined using the second approximation corresponding to the theory of Allegra.[42] These values for representative cases were compared with values obtained using an exact theory developed by Lehman and McTague[43] for finite chains. The values of $\theta_{h,theor}$ obtained from these calculations are shown in Table III along with the original experimental data ($\theta_{h,exptl}$) for comparison.[44] Both the second-order (Allegra) and the first-order (Lifson) approximations give results which agree with those obtained from the exact method. The higher order Allegra approximation will be used in all subsequent discussion of the lysine parameters.

The copolymer melting data were analyzed by assuming that σ is independent of temperature. The best value of σ was obtained by application of the "goodness of fit" criterion defined in paper 2[18] and expressed in terms of the parameter τ. The best fit for all copolymer melting data was obtained by minimizing τ. Figure 5 shows that, in this case, there is no minimum in τ, and that values of σ below 1×10^{-4} fit equally

Table IV
Values of the Zimm–Bragg Parameters s for Poly(L-lysine) in Water or 0.1 N KCl at Neutral pH[a]

Temp, °C	s Lifson	Allegra
0	0.832	0.857
10	0.899	0.909
20	0.934	0.939
30	0.944	0.947
40	0.937	0.939
50	0.924	0.926
60	0.909	0.911

[a] Calculated with $\sigma = 1.0 \times 10^{-4}$.

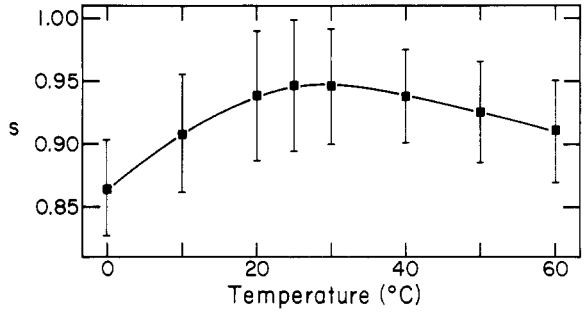

Figure 6. Temperature dependence of s for poly(L-lysine) in water at neutral pH for $\sigma = 1 \times 10^{-4}$. The error symbols are described in section IIIA.

well. Therefore, the maximum value of 1×10^{-4} was arbitrarily chosen as the "best" σ. For $\sigma < 1 \times 10^{-4}$ the computed values of s are found to be independent of σ; for values of $\sigma > 1 \times 10^{-4}$, however, the computed values of s do depend on σ. Since these larger values of σ correspond to a larger τ value, they are thus not applicable. This problem of indeterminacy of σ has been encountered in earlier papers whenever $s < 1.0$, viz., for glycine,[19] serine,[21] and valine.[22] This behavior arises because the low composition of guest residues in the copolymers makes nucleation of helical regions in a pure-guest region of the *random* copolymer a very improbable event; even in a homopolymer, the ratio of the number of residues initiating helical sequences to the number in helical sequences at the melting temperature is about $\sigma^{1/2}$, and the probability of a dilute guest component in a copolymer initiating a helical sequence is much smaller.

The values of $s(T)$ obtained from both the Lifson and the Allegra theories are listed in Table IV. Figure 6 shows the temperature dependence of s with the estimated error in s. The error symbols on the computed values of s are standard deviations on s at a given temperature, calculated from the values of s determined by fitting each fraction individually at that particular temperature using a fixed value of σ. The values of s calculated fo individual fractions were not found to vary in any regular way with composition, implying that there were no discernible lysine–lysine interactions in these copolymers.

The melting curves computed with the best-fit Allegra values for σ and s are shown in Figure 7 along with the experimentally determined points. The error symbols on the computed curves arise from errors in composition (±5–8%) and errors in determination of chain length (molecular weight ±5%). The agreement between the calculated and experimental values of θ_h is reasonably good in most cases.

The thermodynamic quantity $\Delta G°$ for the conversion of an L-lysine residue in a coil state to a helical state at the end of

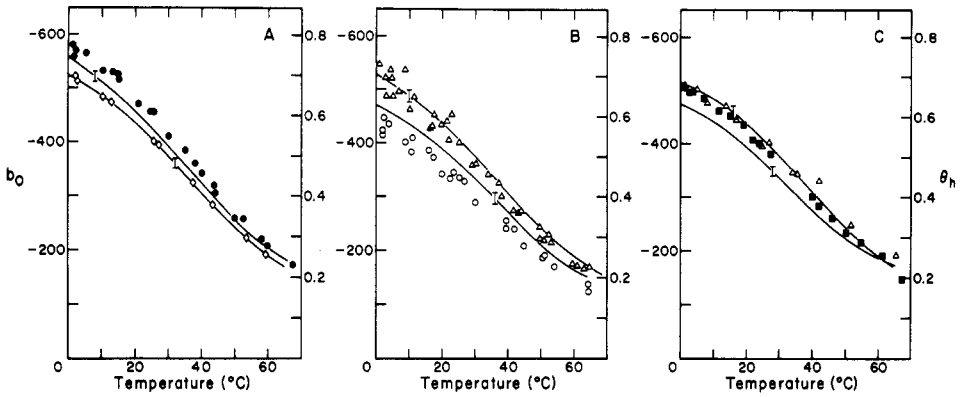

Figure 7. Comparison of the calculated melting curves, obtained with the parameters of the Allegra theory for L-lysine, with the experimental points: (A) (●) 4.7% Lys, $\overline{DP}_w$ = 645 (fraction VIC); (◇) 5.1% Lys, $\overline{DP}_w$ = 370 (fraction VIF); (B) (○) 8.5% Lys, $\overline{DP}_w$ = 230 (fraction VIIID); (△) 8.3% Lys, $\overline{DP}_w$ = 640 (fraction VIIIB); (C) (▲) 5.5% Lys, $\overline{DP}_w$ = 200 (fraction VIIB); (■) 9.7% Lys, $\overline{DP}_w$ = 820 (fraction IXB). The error symbols arise from errors in composition and chain length.

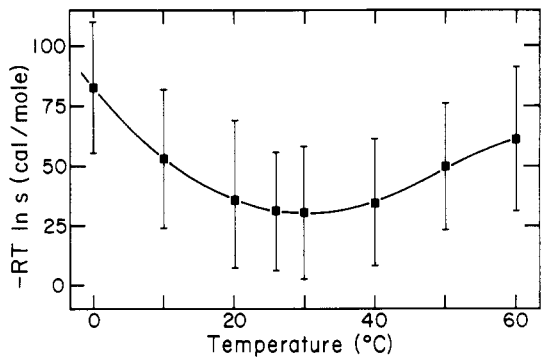

Figure 8. A plot of $-RT \ln s$ vs. T for poly(L-lysine) in water (neutral pH) for $\sigma = 1 \times 10^{-4}$. The error symbols were calculated as in section IIIA.

a long helical sequence can be obtained directly from s (i.e., $\Delta G° = -RT \ln s$). The quantities $\Delta H°$ and $\Delta S°$ for this process can be determined from the temperature dependence of $\Delta G°$ (or s). Figure 8 shows a plot of $\Delta G°$ ($-RT \ln s$) vs. temperature with error symbols calculated from the standard deviations in s. The van't Hoff equation $[-dR \ln s/d(1/T) = \Delta H°]$ was used to determine $\Delta H°_{20}$ from the slope of the ln s vs. $1/T$ curve at 20 °C. The value of $\Delta H°_{20}$ is listed in Table V together with $\Delta S°_{20}$ calculated directly from the relation $\Delta G = \Delta H - T\Delta S$. The $\Delta G°$ vs. temperature curve, as well as the $-R \ln s$ vs. $1/T$ curve, has a minimum in the temperature range 20–30 °C. In this region where the slope is very small, any small variation in the value of $\Delta G°$ (or s) has a large effect on the computed value of $\Delta H°$. Therefore, the values of $\Delta H°_{20}$ and $\Delta S°_{20}$ presented in Table V should be considered only as rough estimates of these quantities.

(B) Comparison with Previous Results. All previous quantitative measurements of σ and s for poly(L-lysine) pertain to lysine in an uncharged state. Because of the observed instability of the copolymers used in the present study at high pH (where the ϵ-amino groups are uncharged), these parameters have been obtained for a charged residue only. It has been demonstrated in a similar study, using the host–guest technique to obtain σ and s for glutamic acid,[16] that there are significant differences between the parameters for a charged and an uncharged residue. For this reason, the parameters obtained in the present work cannot be compared to any previous results. Our results, using the host–guest technique, are unique in that they provide a measure of the intrinsic conformational preference of a charged lysine residue.

A number of copolymers (both random and regular sequence) containing lysine as one of the components have been studied.[45–48] In every case considered, the lysine content exceeded 20 mol %, and the helix content at neutral pH was shown to increase with increasing salt concentration. This behavior implies that long-range electrostatic repulsions between the charged lysine side chains were influencing the conformation. The presence of long-range electrostatic interactions in these polypeptides render them unsuitable for obtaining even a qualitative estimate of the conformational preference of an isolated lysine residue.

The intrinsic helical stability of an amino acid residue has been shown to correlate well with the probability that it will be found in a helical region in proteins.[49–51] Since the ϵ-amino group of lysine is charged at physiological pH, the σ and s parameters determined in this paper are expected to agree more closely with the behavior anticipated from examination of proteins than the parameters previously determined[11–13] for an uncharged lysine. The temperature dependence of s shown in Figure 6 (or of $\Delta G°$ shown in Figure 8) indicates that the helix disrupting ability of a charged lysine residue passes through a minimum at 20–25 °C. Thus, while charged lysine residues appear to prefer the random coil conformation at most temperatures, in the temperature range 20–25 °C where s approaches 1.0, these residues are helix indifferent. Several studies of frequencies of occurrence of amino acids in various conformational regions of proteins of known structure have revealed a *slight* preference for lysine residues to be in α-helical regions. For example, Kotelchuck and Scheraga[52] assigned lysine as a helix breaker, and Burgess et al,[53] Chou and Fasman,[49] and Tanaka and Scheraga[51] classified lysine as one of the weakest helix formers. Thus, the results found here from the random copolymer (in which the near-neighbor interactions are dominant), viz., that s is slightly smaller than 1.0 at room temperature, differ only slightly from conclusions from protein x-ray structures, where longer-range interactions might be expected to play a role to some extent. In fact, it has been shown[50] that, for all charged amino acids (including lysine), the presence of an oppositely charged residue four residues away along the chain enhances its probability for being helical.[54–57] Thus, the study of the random copolymer provides information about the intrinsic tendency of a residue to be helical (based on short-range interactions), and a comparison of this behavior with that in proteins (involving specific sequences) provides information about the medium- and longer-range interactions.

IV. Conclusions

Water-soluble random copolymers containing L-lysine and N^5-(4-hydroxybutyl)-L-glutamine were synthesized and

Table V
Thermodynamic Parameters for L-Lysine[a]

$\Delta G°_{20}$, cal/mol	36 ± 30
$\Delta H°_{20}$, cal/mol	330 ± 210
$\Delta S°_{20}$, eu	1.0 ± 0.5
σ	1×10^{-4}

[a] Calculated as described in text.

characterized. Based on the value of s at 20 °C, it has been concluded that, in the absence of long-range electrostatic repulsions, an isolated charged lysine residue is indifferent as a helix maker. At both higher and lower tempperatures this residue was found to destabilize α helices. It has been shown that, in order to measure the intrinsic conformational preference of charged residues, these residues must be sufficiently isolated from one another so that long-range electrostatic repulsions do not influence the conformation. The host–guest method appears to be the only known method for obtaining these conditions.

Acknowledgment. We are indebted to Mr. H. Chan and Mr. T. Tannhauser for performing the nitrogen and amino acid analyses, and to Mr. G. Davenport for excellent technical assistance. We also thank Dr. A. Ali for synthesis of a sample of α-benzyloxycarbonyl-*N*-ϵ-*tert*-butyloxycarbonyl-L-lysine.

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