Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. VIII. Valine Parameters from Random Poly(hydroxypropylglutamine-*co*-L-valine) and Poly(hydroxybutylglutamine-*co*-L-valine)[1]

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ABSTRACT: The synthesis and characterization of water-soluble "random" copolymers containing L-valine with either N^5-(3-hydroxypropyl)-L-glutamine or N^5-(4-hydroxybutyl)-L-glutamine are described, and the thermally induced helix–coil transitions of these copolymers in water have been studied. The incorporation of L-valine was found to decrease the helix content of the polymer at low temperatures and increase it at high temperatures. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-valine) in water were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers.

This is a continuation of previous papers[3-9] of this series in which random copolymers are used [in the "host–guest" technique with either N^5-(3-hydroxypropyl)-L-glutamine (HPG) or N^5-(4-hydroxybutyl)-L-glutamine (HBG) as the host residue] for the evaluation of the helix–coil stability constants of the naturally occurring amino acids in water. The technique is applied here to obtain the Zimm–Bragg parameters[10] σ and s for L-valine.

It has previously been shown qualitatively that (in organic solvents) L-valine reduces the stability of α-helices in a random[11] copolymer, and either increases or decreases the stability of α helices (depending on the amino acid sequence) in regular-sequence[12] copolymers; in block copolymers,[13] poly(L-valine) assumes a β structure in water but an α-helical one in methanol. The results described here confirm the earlier qualitative work that poly(L-valine) is nonhelical in water (at least at low temperature), and also provide a quantitative description (in terms of σ and s) of the conformational preference of L-valine in water over the temperature range of 0–70°.

The synthesis of water-soluble random copolymers of L-valine with either HPG or HBG is described in section I, and the experimental characterization of these copolymers and their melting behavior in water are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[3] to determine the helix–coil stability parameters of L-valine in water.

I. Experimental Section: Preparation and Characterization of the Copolymers

The copolymers were prepared by first copolymerizing the *N*-carboxyanhydrides of L-valine and γ-benzyl L-glutamate in dioxane with triethylamine as an initiator. The benzyl blocking group was then replaced by reaction with hydroxypropylamine or hydroxybutylamine.

A. Materials. 3-Amino-1-propanol from Aldrich Chemical Co., Inc., and 4-amino-1-butanol from Chemical Procurement Laboratories, Inc., were dried over barium oxide and distilled under reduced pressure. Dioxane was purified shortly before use by refluxing and distilling over sodium. Hexane was dried over calcium sulfate and decanted just before use. Triethylamine was refluxed and distilled with acetic anhydride, and then dried and distilled over KOH. Ethyl acetate was dried over molecular sieves (4A) and decanted just before use. Purified grade dichloroacetic acid was purchased from Fisher Scientific Co. and used without further purification. Anhydrous methyl alcohol and ether from Mallinckrodt Chemical Works were of analytical reagent grade. 2,2,2-Trifluoroethanol obtained from Aldrich Chemical Co., Inc., was stirred over sodium bicarbonate and distilled. L-Valine and L-glutamic acid were purchased from Aldrich Chemical Co., Inc., and L-leucine from Fisher Scientific Co.

Using the L-leucyl dipeptide method of Manning and Moore,[14] the starting L-amino acids were found to contain no detectable amounts (within 0.1%) of D residues.

PHPG of degree of polymerization $\overline{DP}_w$ = 700, and PHBG of $\overline{DP}_w$ = 720, were fraction IIIB and sample V, respectively, of paper II of this series.[4]

B. Synthesis. *N*-Carboxyanhydrides. L-Valine *N*-carboxyanhydride was prepared by the action of phosgene on a suspension of the amino acid in dioxane for several hours at 45–50° as described by Hirschmann *et al.*[15] Several recrystallizations from ether–hexane gave a product with a melting point of 67–68°. The reaction gave a yield of 80%. γ-Benzyl L-glutamate *N*-carboxyanhydride was prepared as in paper IV[6] of this series.

Poly(γ-benzyl-L-Glu$^{m'}$:L-Val$^{n'}$), [P(BzG:Val)], Copolymers I–IV. Random copolymers of L-valine and γ-benzyl L-glutamate containing from 3 to 11% valine were synthesized by polymerization of the carboxyanhydrides in dioxane with triethylamine as an initiator. The two carboxyanhydrides were dissolved in dioxane (at a concentration of about 10 mmol of total carboxyanhydride/100 ml of solvent for copolymers I–III and 10 mmol of total carboxyanhydride/80 ml of solvent for copolymer IV) in the molar ratio desired for the copolymer product. Triethylamine initiator was added to give an A:I ratio of 25 for I–III and 12.5 for IV. The reaction flask was sealed with a "Drierite" drying tube and allowed to stand at room temperature for 5 days for copolymers I–III and only three hours for copolymer IV. The very viscous reaction mixture was then introduced into 500 ml of vigorously stirred absolute ethanol. The white precipitate was collected on a filter funnel, washed thoroughly with ethanol, and dried over P_2O_5 *in vacuo*. The yields ranged from 80 to 45%. The compositions and chain lengths (determined, roughly, with the relationship of Fujita *et al.*[16]) of these copolymers are given in Table I.

Poly[N^5-(3-hydroxypropyl)-L-Gln^m:L-Val^n], [P(HPG:Val)],

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Table I
Compositions and Chain Lengths of the Unfractionated P(BzG:Val) Copolymers

Polymer No.	L-Val Content of Reaction Mix. (mol %)	L-Val Content Found (mol %)	Av Mol Wt[a] × 10^{-3}	$\overline{DP}$
I	10	3.4	410	1900
II	20	6.4	280	1300
III	30	11.0	290	1400
IV	20	8.4	195	930

[a] By viscometry, using the relation of Fujita *et al.*[16] for polymers in $HCCl_2COOH$.

Table II
Characterization of the Fractionated Copolymers

Fraction[a]	L-Val Content (mol %)	$\bar{v}$ (cm³/g)	$\overline{M}_w$ × 10^{-3} [b]	$\overline{M}_z/\overline{M}_w$[c]	$\overline{DP}_w$
VB	3.2	0.791	187	1.00	1020
VIA	5.0				
VIB	5.9	0.817	204	1.11	1050
VIC	6.1				
VID	6.7	0.817	83		430
VIE	7.7				
VIIB	10.9	0.794	102	1.16	580
VIIC	11.3	0.794	71	1.02	400
VIIIB	5.4	0.792	176	1.15	970
VIIIE	7.2	0.793	77.5	1.10	430

[a] Samples V–VIII were obtained from unfractionated I–IV, respectively. The host was HPG except for polymer VI for which HBG was used. The letter corresponds to the fraction obtained in the fractionation procedure. [b] This value was determined by conventional sedimentation equilibrium (with an extrapolation to zero concentration). [c] These measurements were made only for those fractions whose melting curves were determined.

and Poly[N^5-(4-hydroxybutyl)-L-Gln^m:L-Val^n], [P(HBG:Val)], Copolymers V–VIII. The γ-benzyl L-glutamate:L-valine copolymers were treated with 3-amino-1-propanol or 4-amino-1-butanol to yield a series of water-soluble copolymers. The initial products I–IV were treated, as previously described,[6] to yield samples V–VIII. The yields ranged from 80 to 90%.

D. Rate of Incorporation of Valine in Copolymers. In order to determine the rate of incorporation of valine during the polymerization, the amino acid compositions of a P(BzG:Val) copolymer was determined at various stages of the polymerization reaction. Using the same conditions as in the preparation of copolymer I, except that the A:I ratio was 12.5, samples were withdrawn from the reaction mixture at various times over a period of 4 days and added to dilute (~0.1 *M*) HCl in ethanol to decompose the unreacted *N*-carboxyanhydrides and precipitate the polymer that had formed. The white fibrous precipitates were washed with ethanol and air-dried. Each sample was hydrolyzed in 12 *N* HCl at 105° in a sealed ampoule for 4 days, and its amino acid composition was determined on a Technicon autoanalyzer. No peaks other than those of glutamic acid and valine were observed on the chromatogram.

E. Characterization of the Copolymers. The copolymers were characterized as described in paper II[4] of this series. The spectra of aqueous solutions of copolymers V–VIII showed no absorption at 257 mμ, indicating that the benzyl groups were completely removed (within 1.0%) in the final stage of synthesis. The fractionation of copolymers V–VIII in methanol and ether, and the determination of concentrations, viscosities, molecular weights (by equilibrium ultracentrifugation), and optical rotatory dispersion (ORD) and circular dichroism (CD) behavior on selected fractions were carried out as described previously.[4] The amino acid composition of copolymers V–VIII was determined by the same procedure described in section ID, except that hydrolysis was carried out for 1–5 days on one sample to test for complete hydrolysis.

Table III
Time Dependence of Valine Incorporation in Copolymer[a]

Reaction Time (hr)	L-Val Content Found (mol %)
0	9.8[b]
1	2.1
3	3.7
4	5.5
8	7.2
24	8.1
48[c]	
96[c]	

[a] See section ID for experimental details. [b] Composition of initial reaction mixture. [c] These samples could not be hydrolyzed completely under the conditions used.

Analysis of amino acid standards subjected to the same hydrolysis conditions showed that no correction was necessary for the destruction of these amino acids. The average error in the determination of the amino acid compositions was estimated to be ±5%, and that in the molecular weight to be ±6%. The estimated errors in concentration, b_0 of the complete helix and complete coil, and slope of the Moffitt–Yang plot, are ±3%, ±3% and ±300/b_0%, respectively; these errors result in errors in helix contents θ_h (defined as $-b_0/750$) which are shown by error symbols on graphs of θ_h *vs.* temperature.

The starting materials as well as the final copolymers were checked for racemization by the L-leucyl dipeptide method of Manning and Moore.[14] The L-Leu-L-Val and L-Leu-D-Val were separated on a Technicon autoanalyzer with a sodium citrate elution buffer of pH 3.80. Dipeptide standards were prepared from L,D-valine.

II. Results

A. Characterization of the Copolymers. Table I summarizes the compositions and the average degrees of polymerization ($\overline{DP}$) of the unfractionated P(BzG:Val) copolymers, and Table II shows the data for the fractionated copolymers P(HPG:Val) and P(HBG:Val) that were investigated. The usual decrease in $\overline{DP}_w$, attributed to transaminolysis[4-9,17] upon conversion of these polymers to their hydroxyalkylglutamine derivatives, is apparent from a comparison of the two tables.

The first copolymers containing valine that were prepared were I–III. From Table I, it can be seen that only a small fraction (~⅓) of the valine in the reaction mixture appeared as polymer. Increasing the valine concentration in the reaction mixture to obtain copolymers with higher valine content gave solutions which became viscous in a few hours and gelled in 18–24 hr; these polymers (in contrast to samples I–III) could not be hydrolyzed completely in 12 *N* HCl at 105° even after 2 weeks. All of this behavior suggests that valine *N*-carboxyanhydride may polymerize at a smaller rate than benzyl glutamate *N*-carboxyanhydride, leading to polymers which become rich in valine only near the end of the reaction (N-terminal part of chain). Therefore the rate of incorporation of valine during polymerization was measured, and the results are shown in Table III. It can be seen that the amount of valine incorporated increases with reaction time. It thus appears that the valine is not incorporated in the copolymer randomly. In order to check whether the departures from randomness affected the melting behavior, copolymer IV was prepared under the different conditions cited in section IB (with the polymerization reaction terminated after 3 hr). Subsequent analysis (see section III) showed that the melting behavior of fractions derived from polymer IV were similar to those from I to III. This is not unexpected

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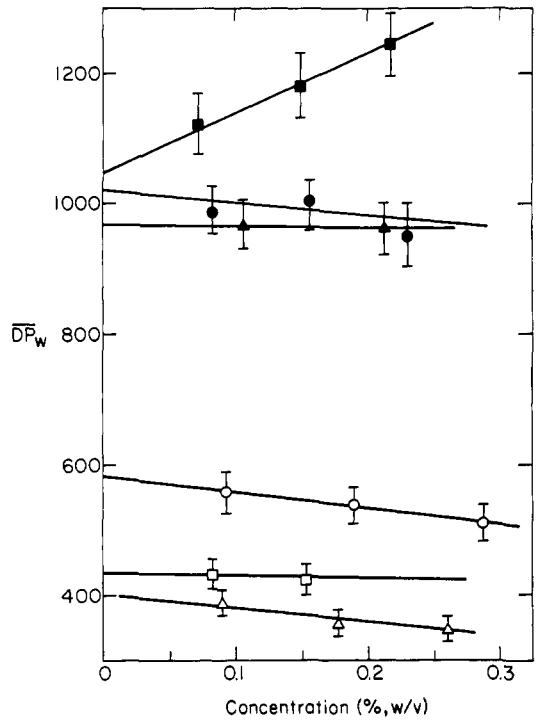

Figure 1. Concentration dependence of molecular weights for fractions used for analysis to obtain σ and s: (■) 5.9% Val (with HBG), $\overline{DP}_w$ = 1050 (fraction VIB); (●) 3.2% Val (with HPG), $\overline{DP}_w$ = 1020 (fraction VB); (▲) 5.4% Val (with HPG), $\overline{DP}_w$ = 970 (fraction VIIIB); (○) 10.9% Val (with HPG), $\overline{DP}_w$ = 580 (fraction VIIB); (□) 7.2% Val (with HPG), $\overline{DP}_w$ = 430 (fraction VIIIE); (△) 11.3% Val (with HPG), $\overline{DP}_w$ = 400 (fraction VIIC). The error symbols represent the experimental error in each measurement.

since it was shown in paper I[3] of this series that fairly large deviations from randomness have little effect on the resulting values of σ and s. This is especially evident in Figure 4 of paper I where the melting curves of poly($A_{\kappa/2}$-$B_{\kappa/2}$) are shown to be insensitive to long runs of A and B, for κ between 10 and 320; this behavior (which holds when s for the guest and host residues are both close to unity near the transition temperature of the copolymer) is exhibited by "random" copolyamino acids with small values of the enthalpy of transition, but not for random copolynucleotides where the enthalpies are much larger. In essence, nonrandom blocks of up to 160 residues could be present in these polymers without influencing their melting behavior; however, it is not likely that such long blocks would be present in chains having the $\overline{DP}_w$ values listed in Table II. Thus, we have analyzed the melting data of fractions V–VIII with a theory[3] based on a random distribution of the host and guest residues in the copolymer.

From Figure 1, it can be seen that five of the six fractions used for the determination of σ and s (in section III) exhibit very little concentration dependence of their $\overline{DP}_w$ values, with the exception of fraction VIB which shows a positive slope. Because the molecular weight of this fraction was high and the fraction was not very heterogeneous ($\overline{M}_z/\overline{M}_w$ = 1.11), it was included with the others in the determination of σ and s.

Using the Manning-Moore[14] dipeptide procedure, the starting materials, *i.e.*, L-valine and L-glutamic acid, were found to contain no detectable D residues to within ±0.1%. Copolymer fraction VIIIE with 7.2 mol % valine and a $\overline{DP}_w$ of 430 was hydrolyzed, and the L-Leu dipeptides were then prepared from the hydrolyzate. Analysis showed that 1.5 mol % of the 31 valines (or only 0.4 residue) were D residues. No racemization of the glutamic acid of the polymer (within ±0.1%) was detected by this procedure. We consider this amount too small to affect the computed values of σ and s for L-valine.

B. ORD and CD Data for the Copolymers. The ORD and CD data for representative samples of fractions of P(HPG:Val) and P(HBG:Val) in water are shown in Figure 2. Both the ORD and the CD data clearly indicate the presence of a right-handed α-helical structure.[18-20] The helix content is seen to increase with decreasing amounts of valine and decreasing temperature. In addition, at high temperature and high valine contents, the polymers appear to be mixtures of helix and random coil with no β structure being detectable,[18-20] indicating that these copolymers undergo a thermally induced transition from the α helix to the random coil in water.

Measurements of b_0, shown in Figure 3 for the six fractions studied over the range of λ = 280 to 420 mμ as a function of temperature, confirm the above conclusion. The procedures used to obtain these curves were the same as those in paper IV.[6] No significant concentration dependence was observed, and the curves were reproducible. The size of the error symbols in Figure 3 reflects the experimental errors in θ_h arising from errors in concentration, in b_0 for the full helix and coil, and in the slope of the Moffitt-Yang plot. The transitions were demonstrated to be reversible in all cases.

These thermally induced melting curves demonstrate several things. First, L-valine is a helix breaker at low temperatures; every melting curve for a copolymer fraction is seen to lie below the corresponding one for the homopolymer. Secondly, at higher temperature[21] L-valine seems to become a helix former. The quantitative analysis in section III will confirm these observations.

C. b_0 for Complete Helix and Complete Coil. For the homopolymers PHBG and PHPG studied in paper II,[4] the value of b_0 for the complete helix was taken to be −750 and for the complete coil as zero. Because these values vary with the nature of the side chain,[22] one fraction with high valine content (VIIC, 11.3 mol % valine) was examined in trifluoroethanol, with b_0 corrected for the dispersion of the refractive index of the solvent.[23] A value of b_0 = −774 was obtained, in close agreement with the above value, and in good agreement with measurements made on other copolymers in this series.[4-9] Thus, −750 was chosen as the value for the complete helix; because of the similarity of these copolymers to the others studied, b_0 = 0 was chosen as the value for the complete random coil.

III. Discussion

A. Helix-Coil Parameters for Poly(L-valine). The melting curves of the copolymers described in section II were analyzed according to the LAPS (Lifson-Allegra-Poland-Scheraga) hierarchy to obtain σ and s for poly(L-valine). This procedure has been discussed extensively in earlier papers of this series.[3-5] To conserve computer time, the first approximation, corresponding to the theory of Lifson[24] was used initially; then better values of σ and s were obtained with the second approximation, corre-

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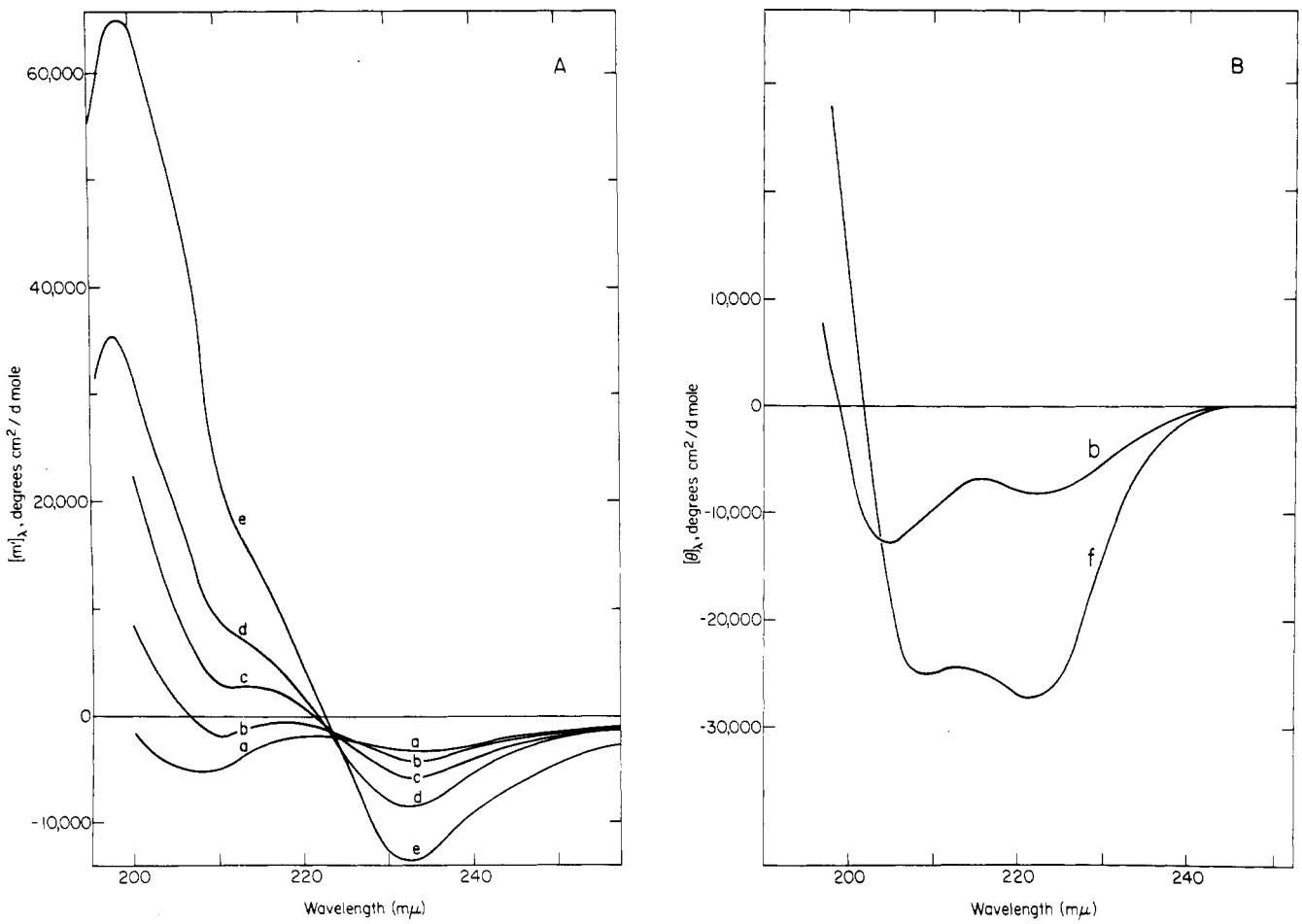

Figure 2. (A) ORD and (B) CD data in water for representative samples of valine copolymers: (a) 7.2% Val (with HPG), $\overline{DP}_w$ = 430 (fraction VIIIE) at 75°; (b) 7.2% Val (with HPG), $\overline{DP}_w$ = 430 (fraction VIIIE) at 25°; (c) 11.3% Val (with HPG), $\overline{DP}_w$ = 400 (fraction VIIC) at 5°; (d) 3.2% Val (with HPG), $\overline{DP}_w$ = 1020 (fraction VB) at 5°; (e) 5.9% Val (with HBG), $\overline{DP}_w$ = 1050 (fraction VIB) at 25°; (f) 6.7% Val (with HBG), $\overline{DP}_w$ = 430 (fraction VID) at 25°.

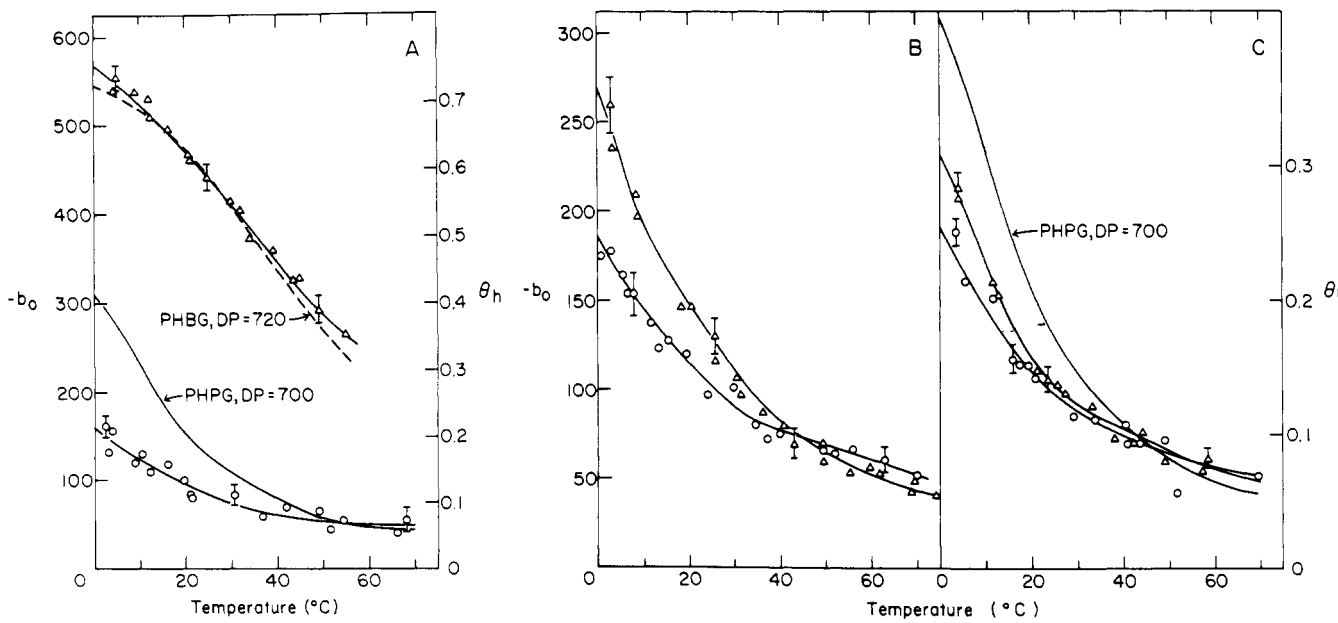

Figure 3. Temperature dependence of b_0 for Val copolymers in water. PHPG of $\overline{DP}_w$ = 700 (fraction IIIB of paper II[4]) and PHBG of $\overline{DP}_w$ = 720 (fraction V of paper II) are included for comparison: (A) (Δ) 5.9% Val (with HBG), $\overline{DP}_w$ = 1050 (fraction VIB); (○) 11.3% Val (with HPG), $\overline{DP}_w$ = 400 (fraction VIIC); (B) (Δ) 3.2% Val (with HPG), $\overline{DP}_w$ = 1020 (fraction VB); (○) 10.9% Val (with HPG), $\overline{DP}_w$ = 580 (fraction VIIB); (C) (Δ) 5.4% Val (with HPG), $\overline{DP}_w$ = 970 (fraction VIIB); (○) 7.2% Val (with HPG), $\overline{DP}_w$ = 430 (fraction VIIIE). The points are the experimental ones, and the lines represent the smoothed experimental curves. The size of the error symbols reflects the experimental errors in θ_h arising from errors in the determination of concentration, in the choice of values of b_0 for the full helix and coil conformations, and of the slope of the Moffitt-Yang plot.

Table IV
Comparison of the Value of θ_h, Calculated with the Approximate and Exact Theories[a] for Finite Chains

L-Val Content (mol Fraction)	$\overline{DP}_w$	Temp (°C)	$(\theta_h)_{exp}$	$(\theta_h)_{theor}$ Allegra[b]	$(\theta_h)_{theor}$ Lehman-McTague[b]
0.113	400	0	0.213	0.203	0.202
		30	0.101	0.116	0.116
		60	0.068	0.078	0.078
0.059	1050	0	0.760	0.739	0.738
		30	0.547	0.536	0.535

[a] The parameters used for PHPG and PHBG were those of Table II in paper II.[4] [b] The parameters used for L-valine were obtained by fitting the data by the Allegra theory, as shown in Table V ($\sigma = 1 \times 10^{-4}$).

Table V
Values of the Zimm-Bragg Parameter s for Poly(L-valine) in Water from 0 to 70°[a]

Temp (°C)	s
0	0.85
10	0.90
20	0.93
30	0.97
40	1.00
50	1.03
60	1.06
70	1.09

[a] Computed with the Allegra theory.[25]

Table VI
Thermodynamic Parameters for L-Valine

ΔG°_{20}, cal/mol	40.4
ΔH°, cal/mol	640 ± 140
ΔS°, eu	2.05 ± 0.47
σ	1×10^{-4}

sponding to the theory of Allegra.[25] The data from the Allegra approximation were checked with the exact theory of Lehman and McTague[26] in two representative cases.[27] The results of these calculations are shown in Table IV along with the original experimental data for comparison. Both the second-order (Allegra) and the first-order (Lifson, not shown in Table IV) approximations give results which agree well with those from the Lehman-McTague method. The higher order Allegra approximation will be used in all subsequent discussion of the valine parameters. These approximations appear to hold for the reasons stated in paper I[3] of this series.

The copolymer melting data were analyzed by finding the best value of σ by application of the "goodness of fit" criterion, expressed in terms of the parameter τ defined in paper II.[4] With σ taken as independent of temperature, the best fit of the copolymer data was obtained by minimizing τ. Figure 4 shows that, in this case, there is no minimum in τ, and that values of σ below 1×10^{-4} fit equally well. This same behavior was observed with the other two helix-breaking residues studied by this method, *viz.*, glycine[5] and serine.[7] In the case of valine, the maximum value of 1×10^{-4} was arbitrarily chosen as the

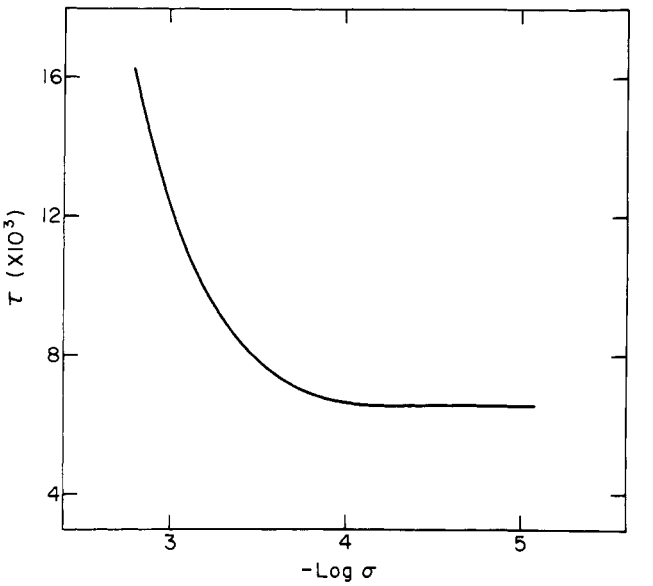

Figure 4. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the valine copolymers, using the Allegra theory.

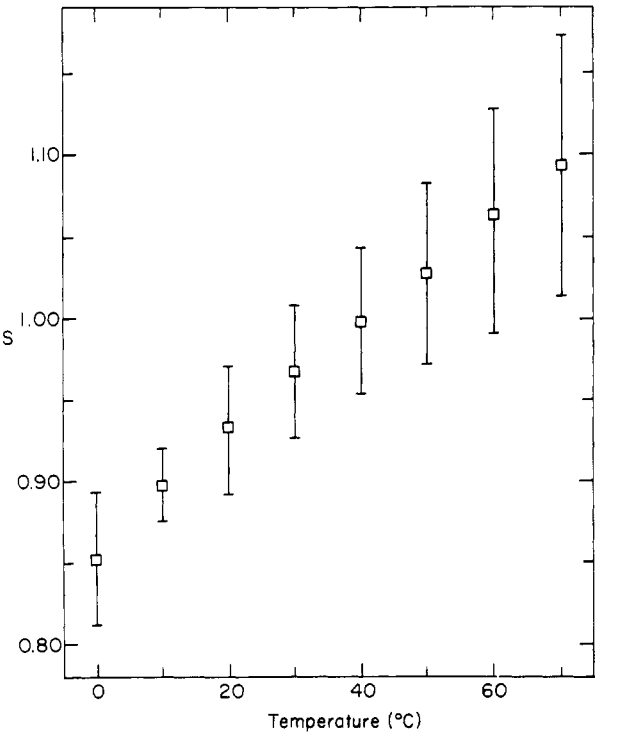

Figure 5. A plot of s *vs.* T for poly(L-valine) in water. The error symbols are described in section IIIA.

"best" σ. The use of values of σ as small as 1×10^{-6} did not alter the computed values of s; while s does depend on σ, for $\sigma > 1 \times 10^{-4}$, such larger values of σ are not applicable since they correspond to larger values of τ. In order to obtain a precise value of the helix-initiating parameter σ, it is necessary to have polymer fractions with a high helix content. Because of the helix-breaking character of valine (as with glycine[5] and serine[7]), all but one of the fractions used here had too low a helix content for us to be able to establish the value of σ more precisely than that given above. Finally, no account was taken of possible errors in the Zimm-Bragg parameters for PHBG and PHPG in assessing the errors in σ and s.

The values of s from 0 to 70° for poly(L-valine) are listed

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(27) All computer programs used in these calculations are available and can be obtained as directed in footnotes 26 and 27 of paper I.[3]

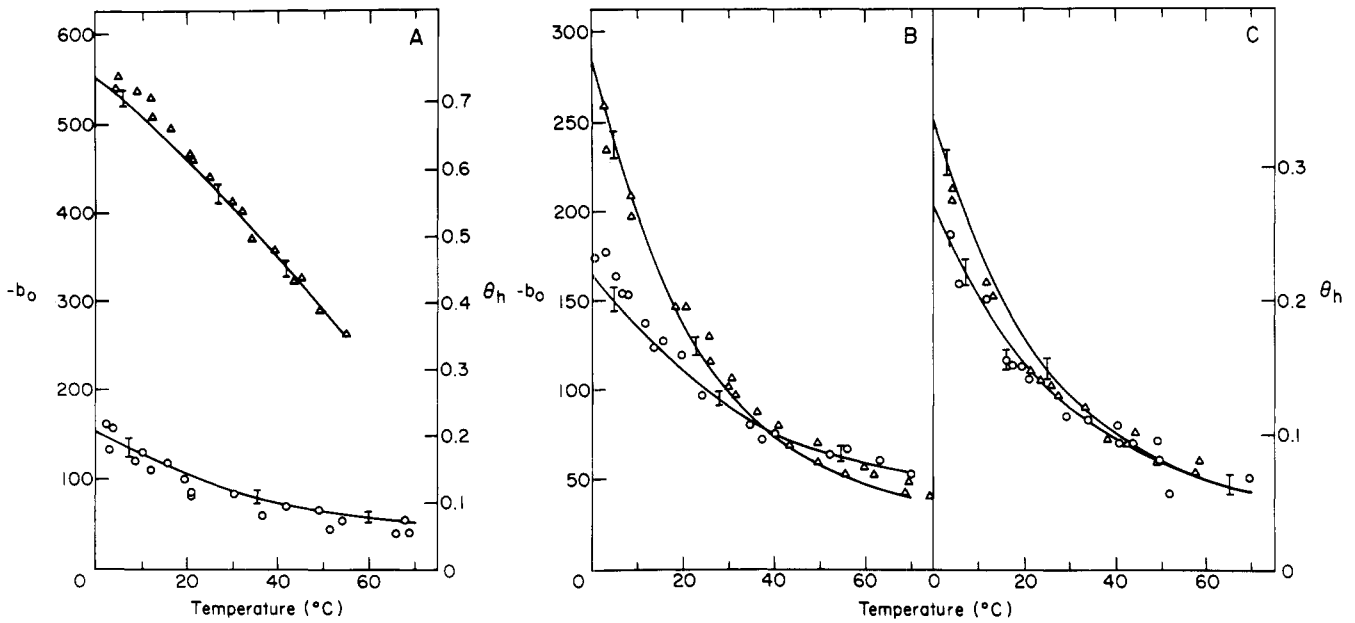

Figure 6. Comparison of the calculated melting curves, obtained from the parameters of the Allegra theory (with $\sigma = 1 \times 10^{-4}$) for L-valine given in Table VI, with the experimental points: (A) (Δ) 5.9% Val (with HBG), $\overline{DP}_w$ = 1050 (fraction VIB); (○) 11.3% Val (with HPG), $\overline{DP}_w$ = 400 (fraction VIIC); (B) (Δ) 3.2% Val (with HPG), $\overline{DP}_w$ = 1020 (fraction VB); (○) 10.9% Val (with HPG), $\overline{DP}_w$ = 580 (fraction VIIB); (C) (Δ) 5.4% Val (with HPG), $\overline{DP}_w$ = 970 (fraction VIIIB); (○) 7.2% Val (with HPG), $\overline{DP}_w$ = 430 (fraction VIIIE). The error symbols indicate errors in the calculated values of θ_h arising from errors in composition and chain length (see Figure 3 for additional errors in experimental points).

in Table V. Figure 5 shows the temperature dependence of s with error limits; the latter were computed by fitting each curve individually with $\sigma = 1 \times 10^{-4}$ and then taking the standard deviation of these values of s at each temperature. Figure 6 shows the computed melting curves (using the best-fit Allegra values) along with the experimental points. The error symbols on the computed curves arise from errors in the amino acid analysis and molecular weight. The agreement between the calculated and experimental values is fairly good. Because of the large uncertainty in σ, it was not felt justified to attempt to fit the data with a temperature-dependent σ.

The thermodynamic quantities[28] $\Delta G°$ (the free energy), $\Delta H°$ (the enthalpy), and $\Delta S°$ (the entropy) for the conversion of a coil residue of L-valine to a helical one at the end of a long helical sequence can be obtained from the values of s and its temperature dependence. Figure 7 shows a plot of $\Delta G°$ (= $-RT \ln s$) *vs.* temperature with error symbols calculated as in Figure 5. The data were fit by a weighted least-squares method, using the deviations in Figure 7 as weighting factors[29] as described in paper IV.[6] The resulting least-squares line is shown in Figure 7. The calculated thermodynamic parameters are listed in Table VI. The deviations listed are those derived from the least-squares fitting procedure on the slope and intercept.

B. Comparison with Other Results. The data obtained here provide a quantitative description (in terms of σ and s), which agrees with the earlier qualitative ones[11,12] in organic solvents that L-valine does not generally tend to be helical at room temperature. The only data for poly(L-valine) *in water*, with which our results can be compared, are those for a block copolymer of L-valine [between two

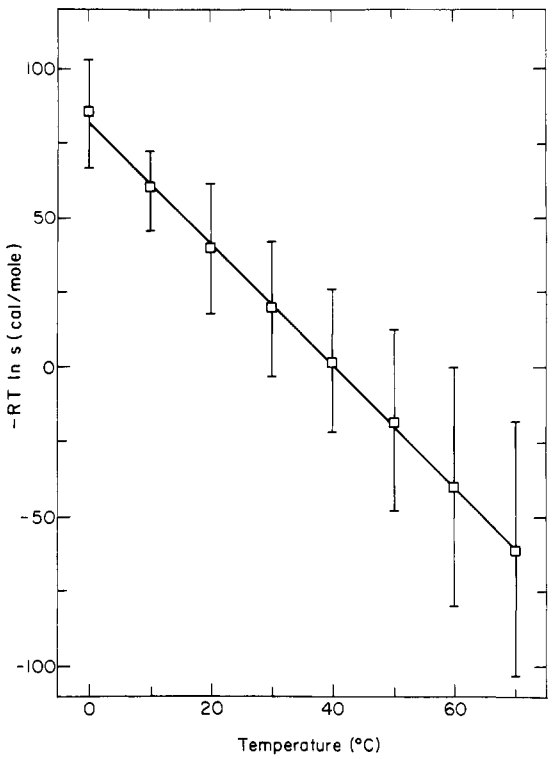

Figure 7. A plot of $-RT \ln s$ (*i.e.*, $\Delta G°$) *vs.* T for poly(L-valine) in water. The points are experimental ones from the Allegra analysis (with $\sigma = 1 \times 10^{-4}$) while the line is the least-squares fit. The error symbols were calculated as described in section IIIA.

blocks of poly(D,L-lysine)].[13] In the block copolymer, L-valine adopts a nonhelical conformation in water; our values of $s < 1$ at room temperature are in agreement with this result. However, the poly(L-valine) blocks did adopt an α-helical conformation in methanol, showing that there is no steric hindrance to α-helix formation from a valine side chain; our values of $s > 1$ at high tempera-

(28) While these thermodynamic quantities are standard-state values, the standard-state symbol has usually been omitted in literature on the helix-coil transition and in previous papers of this series. We include it here with the assumption that the translational and overall rotational entropy are independent of conformation, and that the solvation entropy corresponds to that of a polymer at infinite dilution.

(29) P. R. Bevington, "Data Reduction and Error Analysis for the Physical Scientist," McGraw-Hill Book Co., New York, N. Y., 1969, Chapter 6, p 92.

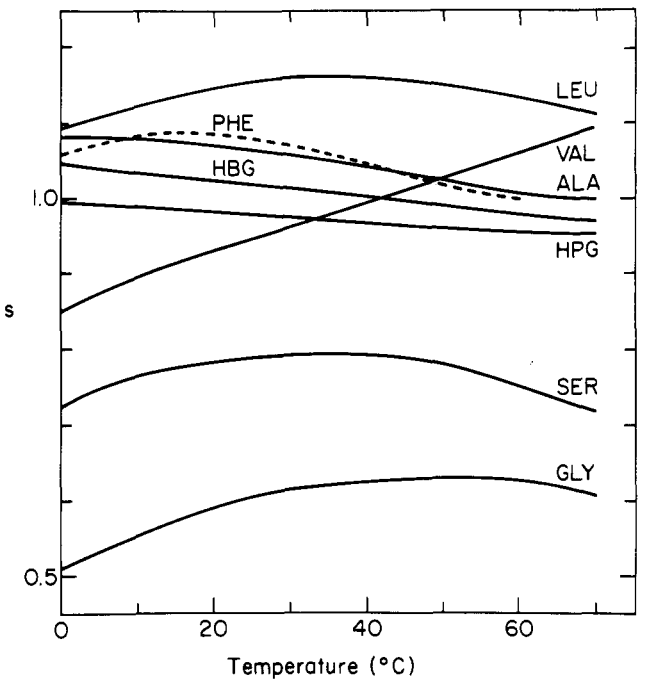

Figure 8. Temperature dependence of s for various amino acid residues in water studied in this series of papers. The sources of the data are given in the text.

ture are in agreement with this result, *i.e.*, that L-valine can fit into an α helix.

The values of $s(T)$ for poly(L-valine) in water have been computed from empirical energy functions,[30] as was done previously[31] for polyglycine and poly(L-alanine). The computed values agree with the experimental ones.

C. Implications. Even though L-valine is a helix breaker in water ($s < 1$) at room temperature (implying that its natural tendency is to adopt a nonhelical conformation), a single L-valine residue (as is the case with any helix breaker)[32,33] can be forced into a helix conformation when it is surrounded by other helix-forming residues in a protein. In fact, valine does appear in helical sections, *e.g.*, in myoglobin[34] and carboxypeptidase.[35] Nevertheless, as a helix breaker at room temperature, valine would tend to lower the stability of any helical section in which it exists.

Figure 8 shows the values of s found in this paper for valine plotted together with those of HPG,[4] HBG,[4] glycine,[5] alanine,[6] serine,[7] leucine,[8] and phenylalanine.[9] Among the nonpolar residues in Figure 8, valine is the only one that is a helix breaker at room temperature, alanine, leucine, and phenylalanine all being helix formers in water.

The positive value of $\Delta G°$ for valine at 20° (see Table VI) arises from a positive $\Delta H°$ which dominates a positive $\Delta S°$. However, at higher temperatures, the entropy term dominates, and $\Delta G°$ becomes negative. For alanine,[6] leucine[8] and phenylalanine,[9] $\Delta H°$ and $\Delta S°$ are both positive and $\Delta G°$ is negative at 20°.

The physical origin of the differences between poly(L-valine) and poly(L-alanine) (in terms of differences between specific interaction energies and hydrophobic bonding in the helix and coil forms, respectively) will be described in ref 30.

IV. Conclusions

Water-soluble "random" copolymers containing L-valine and either N^5-(3-hydroxypropyl)-L-glutamine or N^5-(4-hydroxybutyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transitions of these copolymers, the Zimm–Bragg parameters σ and s for poly(L-valine) were deduced. The values of s show that L-valine is a helix breaker at room temperature but a helix former at higher temperatures. At ~70°, the helix forming ability of valine becomes comparable to that of leucine.

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(31) M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, **54**, 4489 (1971).
(32) D. Kotelchuck and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, **62**, 14 (1969).
(33) P. N. Lewis and H. A. Scheraga, *Arch. Biochem. Biophys.*, **144**, 576 (1971).
(34) H. C. Watson, *Progr. Stereochem.*, **4**, 299 (1969).
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