Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 20. Reinvestigation of Valine Parameters from Random Poly[(hydroxypropyl)glutamine-*co*-L-valine][1]

M. C. Chang, R. A. Fredrickson,[2a] S. P. Powers, and H. A. Scheraga*[2b]

Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853.
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ABSTRACT: Even though L-valine and L-isoleucine are both β-branched amino acids, the effect of temperature on their helix-forming tendency differs markedly. Since this apparent anomaly might have arisen from significant nonrandom incorporation of L-valine into the copolymer used previously to determine its helix–coil stability constants, this copolymer is reinvestigated here, using a different method of synthesis to avoid formation of large, nonrandom blocks of L-valine. Within experimental error, the same temperature dependence of the Zimm–Bragg parameter s for L-valine is obtained as found with the copolymer synthesized in earlier work. The difference in the behavior between L-valine and L-isoleucine is therefore a real one.

The "host–guest" technique[3] makes use of random copolymers to determine the helix–coil stability constants for the naturally occurring amino acids in water. Application of this technique to L-valine[4] and L-isoleucine[5] indicated that these two β-branched amino acids differ considerably in their helix-forming tendency, as expressed by the Zimm–Bragg parameters[6] s and σ. For L-valine, the values of s increase monotonically with increasing temperature[4], whereas those of L-isoleucine decrease.[5] At 20 °C, $s = 0.93$ and $\sigma = 1 \times 10^{-4}$ for L-valine,[4] and $s = 1.05$ and $\sigma = 55 \times 10^{-4}$ for L-isoleucine.[5] Since this difference in behavior might have arisen from the existence of nonrandomly-incorporated blocks of L-valine in the copolymer prepared previously,[4] this problem is reinvestigated here, using a different procedure to synthesize the copolymer. As in the case of the isoleucine-containing copolymer,[5] we take account in the new synthesis here of the large difference in relative reactivities of the *N*-carboxyanhydrides (NCA) of L-valine and γ-benzyl L-glutamate. This is accomplished by periodic additions of the faster reacting Glu(OBzl)-NCA during the polymerization reaction. This procedure should thereby prevent the formation of long blocks of L-valine near the end of the chain.

Experimental Section

The copolymers were synthesized by polymerizing L-valine NCA and γ-benzyl L-glutamate NCA in dioxane, using sodium methoxide as an initiator. The γ-benzyl blocking groups were subsequently exchanged by reaction with hydroxypropylamine to yield poly[N^5-(3-hydroxypropyl)-L-glutamine-*co*-L-valine].[7]

L-Valine was purchased from Aldrich Chemical Co., Inc. All other reagents and solvents were identical in quality and preparation with those used in paper 19 of this series.[5] The synthesis of L-valine NCA is the same as in paper 8 of this series[4]. γ-Benzyl L-glutamate NCA was prepared as described in paper 19 of this series.[5]

Poly(γ-benzyl L-glutamate-*co*-L-valine), Poly[Glu-(OBzl), Val]. L-Val-NCA (30 mol %) and γ-benzyl L-glutamate NCA were dissolved in dioxane (10 mmol total NCA/90 mL). The initiator, sodium methoxide, was added to an A:I molar ratio of 40. The reaction flask was sealed with a drying tube and allowed to stand at room temperature for 60 min before quenching. As described in paper 19,[5] periodic additions of γ-benzyl L-glutamate NCA were made to prevent the depletion of this relatively faster reacting NCA during the course of the polymerization. In an attempt to maintain the initial mole ratio of the two NCA's, aliquots of γ-benzyl L-glutamate NCA were added to the reaction mixture after 20 and 40 min. The randomness of the polymerization was determined by following the composition of the unreacted monomer pool and of the growing polymer chains, as described in paper 19.[5] Table I summarizes the progress of the synthesis. The polymerization was quenched and the copolymer isolated as described in paper 19.[5] After 60 min, the yield was 53%. The chain length was determined roughly by viscometry in DMF, as in paper 19,[5] to be 855, and the overall composition was approximately 6 mol % valine.

Table I
Synthesis of Poly[Glu(OBzl),Val][a]

reaction time, min	composition (mol % L-valine)[b] monomer pool	growing polypeptide chain
0	27.5	
18	30.2	9.5
20	addition of Glu(OBzl)-NCA	
22	23.8	8.6
38	29.3	5.7
40	addition of Glu(OBzl)-NCA	
42	30.5	8.1
58	40.2	6.1
60	reaction quenched	

[a] Mole ratio of anhydride to initiator was 40. [b] Reaction was monitored by quenching 0.5 mL of the reaction mixture with 5 mL of 0.1 N HCl/ethanol.

Table II
Characterization of Fractionated Copolymer

fraction	L-Val content, mol %	$\bar{M}_w \times 10^{-3}$ [a]	$\bar{M}_z/\bar{M}_w$ [b]	$\overline{DP}$ [a]
2	7.0	143.2	1.01	795
4	7.0	74.6	1.05	414
5	6.8	52.6	1.05	292

[a] By conventional sedimentation equilibrium, with an extrapolation to zero concentration. Mean residue weight for fractions 2 and 4 was 180.1 and for fraction 5 was 180.3. [b] $\bar{M}_z/\bar{M}_w$ is reported for the run at lowest concentration.

Poly[N^5-(3-hydroxypropyl)-L-glutamine-*co*-L-valine], Poly[HPG,Val]. The benzyl-protected copolymer was dissolved in dioxane and treated with 3-amino-1-propanol at 50° C under nitrogen to yield a water-soluble copolymer, poly[HPG,Val], as described previously.[5] After the aminolysis reaction, dialysis, and lyophilization were completed, as described in paper 19,[5] the water-soluble copolymer was recovered in 50% yield, based on the number of moles of the benzyl-protected copolymer. The water-soluble copolymer was fractionated by the same procedure used in paper 19 of this series;[5] the fractions were then dissolved in water and lyophilized. The composition and chain length of the fractions that were used for the study of their thermally induced helix–coil transitions are summarized in Table II.

Analytical Methods. Determination of Composition and Concentration and Measurement of Viscosity, Optical Rotatory Dispersion (ORD), Circular Dichroism (CD), and Molecular Weight. Amino acid compositions of the fractions

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Table III
Values of the Zimm-Bragg Parameter s for Poly(L-valine) in Water from 0 to 70 °C

temp, °C	Lifson[a]	Allegra[b]	Allegra[a]	data from ref 4 Allegra[c]
0	0.87	0.87	0.88	0.85
5	0.90	0.90	0.90	
10	0.92	0.92	0.92	0.90
15	0.94	0.93	0.94	
20	0.95	0.95	0.95	0.93
25	0.96	0.96	0.96	
30	0.97	0.96	0.97	0.97
35	0.97	0.98	0.97	
40	0.98	0.97	0.98	1.00
45	0.98	0.97	0.98	
50	0.99	0.98	0.99	1.03
55	1.01	1.00	1.01	
60	1.04	1.02	1.04	1.06
65	1.08	1.07	1.08	
70	1.13	1.18	1.13	1.09

[a] Computed for $\sigma = 1 \times 10^{-5}$, using either the Lifson or Allegra method, as in paper 19.[5] [b] Computed for $\sigma = 1 \times 10^{-4}$, using the Allegra method. [c] Computed for $\sigma = 1 \times 10^{-5}$, using the Allegra method. In the earlier paper, the same values were obtained with $\sigma = 1 \times 10^{-4}$.

were determined by hydrolyzing each fraction in 6 N HCl at 105 °C in evacuated, sealed ampules for 5 days. All other measurements and procedures are identical with those described in paper 19.[5]

Results and Discussion

In an identical test copolymer synthesis of poly[Glu(OBzl),Val], except that Glu(OBzl)-NCA was *not* added periodically, Glu(OBzl)-NCA reacted about 4 times faster than Val-NCA. This behavior presumably led to nonrandom incorporation of valine in the earlier study[4] of such copolymers. Table I indicates that periodic addition of the more reactive NCA prevented a large increase in concentration (in the monomer pool) of the less reactive NCA toward the end of the polymerization. The randomness of the synthesized copolymer was thereby ensured by this technique.

The Lifson–Allegra–Poland–Scheraga (LAPS) hierarchy was used to analyze the melting curves of the copolymer fractions of Table II to determine the Zimm–Bragg parameters[6] σ and s. Table III lists the results of this analysis.

The best value of σ was found to be 1×10^{-5} through application of the "goodness of fit" criterion, expressed in terms of τ, as was done in paper 19.[5] No minimum in τ was found and all values of σ below 1×10^{-5} fit equally well. This behavior has been observed for all helix-breaking residues studied thus far in this series, except for aspartate.

Figure 1 shows the temperature dependence of s for poly(L-valine) as determined from this work and from the original study.[4] The newly computed values of s lie within the error limits determined previously.[4] Therefore, it is concluded that the results which were obtained earlier with

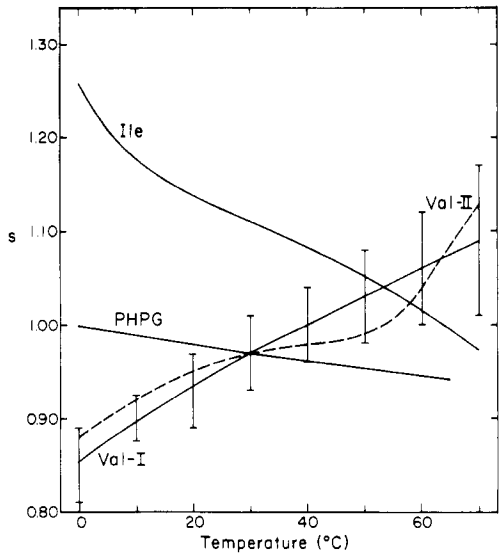

Figure 1. Plot of s vs. T for poly(L-isoleucine),[5] poly(HPG),[7] and poly(L-valine)[4] (Val-I and Val-II). The data for Val-I and its associated error limits were taken from paper 8.[4] The data for Val-II are those obtained in this study.

presumably nonrandom copolymers of L-valine[4] are reproduced with the random copolymers synthesized for this study. The reason for this is that, as shown previously,[3] short blocks of guest residue (~10–15% of $\sigma^{-1/2}$ in length) do not alter the appearance of the transition curve of a random copolymer.

From the characterization and analysis of a water-soluble random copolymer, synthesized with a new method[5] to ensure randomness, it is thus confirmed that L-valine is a helix breaker at low temperatures and a helix maker at higher temperatures. The value of σ indicates that L-valine is a poor helix initiator. The Zimm–Bragg parameters, σ and s, have been found to be similar to those determined in paper 8.[4] L-Valine is thus confirmed to be distinctly different from L-isoleucine in its helix–coil transition behavior.

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References and Notes

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(2) (a) NIH Predoctoral Trainee. (b) Author to whom requests for reprints should be addressed.
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