

COMMUNICATIONS TO THE EDITOR

Helix-Coil Stabilities of L-Alanine and L-Leucine in Mixed Organic Solvents

For several years, we have been engaged in evaluating the helix-coil stability constants for the naturally occurring amino acids in water.¹ Specifically, we have determined the values of the parameters σ and s of the Zimm-Bragg theory² for several amino acids in aqueous solution, using the "host-guest" technique.³ A similar series of studies to determine these same parameters in nonaqueous solvents was initiated by first obtaining σ and s for the host residue, γ -benzyl-L-glutamate [in the form of its homopolymer, poly(γ -benzyl-L-glutamate)], in mixtures of dichloroacetic acid and dichloroethane.⁴ In this communication, we report some of our data on the helix-coil transition of random copolymers containing γ -benzyl-L-glutamate as the host and L-alanine and L-leucine, respectively, as the guest residues, in a mixture of 82 wt% of dichloroacetic acid and 18 wt% of dichloroethane. At this solvent composition, γ -benzyl-L-glutamate can be expected⁴ to be a suitable host for residues that, potentially, are helix-formers.

Random copolymers containing γ -benzyl-L-glutamate and varying amounts of either L-alanine or L-leucine were synthesized using the procedures described earlier.^{5,6} The compositions of these copolymers were determined with an amino acid analyzer, and their approximate molecular weights were estimated from viscosity measurements in dichloroacetic acid, assuming that the viscosity-molecular weight relation of Doty et al.⁷ for poly(γ -benzyl-L-glutamate) would hold for the copolymers as well. The copolymers were fractionated by fractional precipitation in dioxane-ethanol,⁴ and the middle fractions were chosen for the helix-coil transition experiments. The data characterizing the copolymers are summarized in Table I. The helix-coil transition of the fractionated copolymer samples in 82 wt% dichloroacetic acid in the temperature range of 0–70°C was monitored by means of the Moffitt-Yang b_0 parameter.⁸ A Jasco J-20 spectropolarimeter was used for this purpose, and also for obtaining the optical rotatory dispersion and circular dichroism spectra of the copolymers in the ultraviolet region, which indicated no evidence for the presence of β -structure in the copolymers.

The helix-coil transition curves for the copolymer fractions are shown in Fig. 1. For clarity, the data on fractions I-C1 and III-B2 (Table I) are not shown in Fig. 1, since they are very close to those of fractions I-C3 and III-B3, respectively. The transition curve of poly(γ -benzyl-L-glutamate) at the same solvent composition⁴ is also shown in Fig. 1 for comparison. The fractional helix content, θ_h , was calculated from the b_0 data, using -700 and $+75$ for the 100% helix and 100% coil values for b_0 , respectively,⁴ and is shown on the right-hand ordinate of Fig. 1.

The copolymers exhibit the "inverted transition" observed⁹ for poly(γ -benzyl-L-glutamate), with the coil form being more stable at the lower temperatures. The transition is found to be reversible. As expected, the transition curves of the copolymers are shifted towards lower temperatures with respect to that of poly(γ -benzyl-L-glutamate), indicating that the incorporation of either L-alanine or L-leucine increases the stability of the helical conformation of the host polyamino acid. However, in contrast to the behavior in aqueous solution,^{5,6} L-alanine is seen to be a stronger helix-former than L-leucine in the mixed solvent system. The

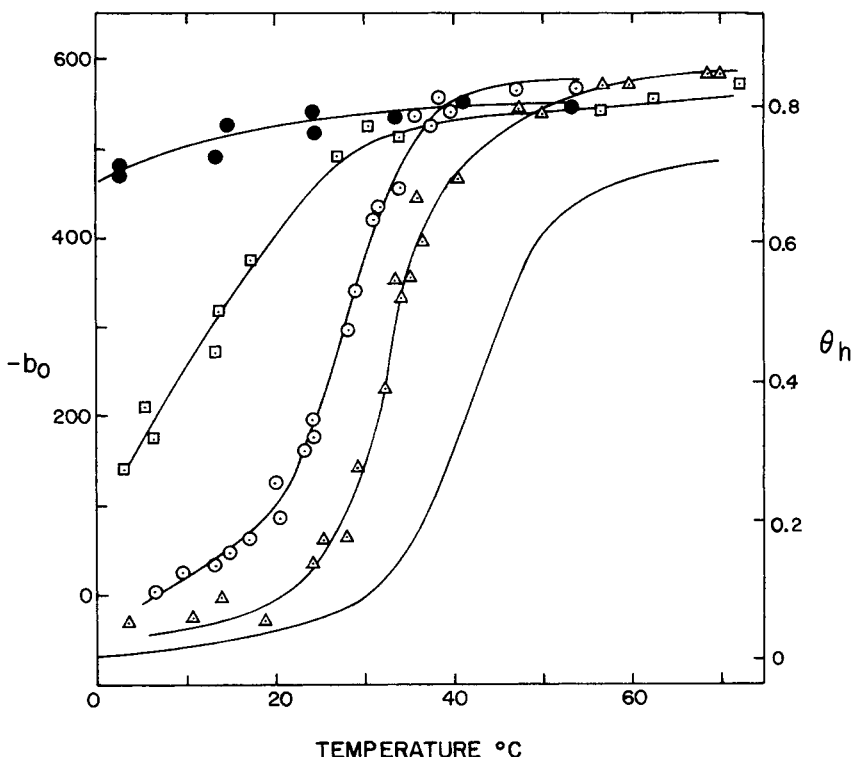


Fig. 1. Helix-coil transition curves for the random copolymers in 82 wt% dichloroacetic acid. ●, Fraction II-B, 31.4% Ala; □, Fraction I-C3, 14.0% Ala; ○, Fraction IV-B1, 13.2% Leu; △, Fraction III-B3, 5.7% Leu. The transition curve for poly(γ -benzyl-L-glutamate), $DP = 1000$ (Fraction II-2 of Ref. 4), is also shown for comparison (curve without points).

transition temperature (given in the last column of Table I) for the copolymer containing 14.0% L-alanine is about 15°C lower than that of the 13.2% L-leucine copolymer and about 32°C lower than that of poly(γ -benzyl-L-glutamate). The copolymer with 31.4% L-alanine remains largely helical even at 0°C. The chain lengths of these copolymers (as determined by viscosity measurements on primarily the unfractionated samples; see Table I) are large (>1300), so that variations of the order of a few hundred units in the chain lengths would be expected to cause no significant change in the transition curves, as was demonstrated earlier in the case of poly(γ -benzyl-L-glutamate).⁴

Using the homopolypeptides, poly(L-alanine) and poly(L-leucine), in a mixed solvent system consisting of chloroform/dichloroacetic acid/trifluoroacetic acid, Fasman¹⁰ observed that poly(L-alanine) was more resistant to the influence of the helix-disrupting solvent, trifluoroacetic acid, than poly(L-leucine). However, the plots of b_0 against the solvent composition for these polyamino acids reported by Fasman¹⁰ cross over each other at two solvent compositions, making it difficult to obtain an unambiguous interpretation of the data. Our data, presented here for different copolymers at identical solvent compositions, show that the L-alanine residue is thermodynamically more stable in the α -helical conformation than the L-leucine residue, in the nonaqueous solvent system employed here. Using a similar approach, viz., the host-guest technique, we have demonstrated earlier that, in water, L-leucine forms a more stable α -helix than L-alanine.^{5,6} The reason for this reversal in the helix stabilities of these two amino acid residues is most likely due to the difference in the interactions of these

TABLE I
Compositions and Chain Lengths of the Random Copolymers of γ -Benzyl-L-Glutamate
with L-Alanine and L-Leucine

Sample No.	Mol%		\bar{M}^a ($\times 10^{-5}$)	Average Chain Length	Transition Temperature (°C)
	L-Ala	L-Leu			
I	14.7		3.06	1545	
I-C1	14.9		3.00	1515	13.0
I-C3	14.0		2.99	1533	13.0
II	30.2		4.79	2732	
II-B	31.4				<0
III		5.2	2.88	1347	
III-B2		4.4			33.5
III-B3		5.7			33.5
IV		17.5	2.98	1497	
IV-B1		13.2			28.5

^a Average molecular weight determined from viscosity measurements in dichloroacetic acid.

amino acid residues with the solvent medium. In aqueous solution, it is conceivable⁶ that the α -helical conformation is more stable for L-leucine than for L-alanine because of stronger hydrophobic interactions involving the leucine side chain, while, in the nonaqueous solvent, unfavorable nonbonded interactions involving the leucine residue may tend to make its helical conformation less stable than that of the alanine residue.

These data indicate that the behavior of amino acid residues in both aqueous and nonaqueous solvents will have to be taken into consideration in accounting for the stabilities of helical segments in globular proteins, since the helices may be either partially or completely buried. Quantitative evaluation of the Zimm-Bragg parameters for the L-alanine and L-leucine residues in the mixed solvent system is now in progress.

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