83831-53-2; β-poly-8, 83831-55-4; α-poly-9, 83831-57-6; β-poly-9, 83831-59-8; α-poly-11, 83831-61-2; β-poly-11, 83831-63-4.

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Helix Initiation and Propagation by (Hydroxyethyl)-L-glutaminyl Residues in Water

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Received March 18, 1982

ABSTRACT: Random copolypeptides composed of (hydroxyethyl)-L-glutaminyl and (hydroxybutyl)-L-glutaminyl residues have been synthesized by aminolysis of high molecular weight poly(γ-benzyl L-glutamate). The mole fraction of (hydroxyethyl)-L-glutaminyl residues in the copolypeptides ranged from 0.37 to 0.81. Circular dichroism spectra measured in water from 4 to 64 °C had the shapes and intensities expected for partially helical polypeptides. Zimm–Bragg statistical weights for the (hydroxyethyl)-L-glutaminyl residue in water were deduced by matrix methods and the σ,s reported by von Dreele et al. (*Macromolecules* **1971**, *4*, 408–417) for the (hydroxybutyl)-L-glutaminyl residue. Ability of theory to reproduce experimental helicities is not sensitive to the value assigned to σ for the (hydroxyethyl)-L-glutaminyl residue so long as that value is small. With $\sigma = 1 \times 10^{-5}$, s for the (hydroxyethyl)-L-glutaminyl residue is found to decrease from 0.945 to 0.928 as the temperature rises from 4 to 64 °C. The observed behavior of the (hydroxyethyl)-L-glutaminyl residue in water is in harmony with the trend established by (hydroxypropyl)-L-glutaminyl and (hydroxybutyl)-L-glutaminyl residues.

The series of homopolypeptides based on (hydroxyalkyl)-L-glutamine has been studied extensively. The three most prominent members of this series are the poly[(hydroxyalkyl)-L-glutamines] with alkyl = ethyl, propyl, and butyl. These three nonionic homopolypeptides exhibit a wide range of conformational properties in aqueous solution. Hydrodynamic[1] and optical[1-3] properties of poly[(hydroxyethyl)-L-glutamine] in water are those expected for a disordered homopolypeptide bearing a CH_2R side chain. In contrast, at low temperatures in water, poly[(hydroxypropyl)-L-glutamine] is partially helical and poly[(hydroxybutyl)-L-glutamine] is predominantly helical.[2,4-8] Poly[bis(hydroxyethyl)-L-glutamine] shows behavior similar to that exhibited by poly[(hydroxypropyl)-L-glutamine].[9] Helical content of these three polypeptides is reduced by an elevation in temperature. Helicity of poly[(hydroxyalkyl)-L-glutamines] can also be modified by changes in solvent composition. Inorganic salts[4,8,10] and organic cosolvents[2,4,5,7,11-16] are effective in this regard.

Zimm–Bragg[17] statistical weights, σ and s, have been determined for (hydroxypropyl)-L-glutaminyl[5-7] and (hydroxybutyl)-L-glutaminyl[6] residues in water by study of the homopolypeptides and also via the "host–guest" technique. While poly[(hydroxyethyl)-L-glutamine] does not present solubility problems in water, its values for σ and s still cannot be determined by study of the homopolypeptide because the observed helicity is vanishingly small. We report here σ and s for the (hydroxyethyl)-L-glutaminyl residue which were determined by the "host–guest" technique, with (hydroxybutyl)-L-glutaminyl residues playing the role of host. These statistical weights are obtained by examining the manner in which helicity declines when (hydroxyethyl)-L-glutaminyl residues are randomly incorporated into poly[(hydroxybutyl)-L-glutamine]. The results provide insight into the conformational consequences of addition of methylene groups to the periphery of an amino acid residue bearing a long nonionic side chain.

Experimental Section

Copolypeptides were prepared in dioxane by aminolysis of poly(γ-benzyl L-glutamate) using a mixture of hydroxyethanolamine and hydroxybutanolamine. Reaction conditions were a slight modification of the procedure by which poly[(hydroxybutyl)-L-glutamine] was prepared from poly(γ-benzyl L-glutamate).[18] Copolypeptide compositions were determined from a quantitative ninhydrin analysis of the hydroxyethanolamine and hydroxybutanolamine in the acid hydrolysate, using a Beckman 119 amino acid analyzer, and also from proton magnetic resonance spectra.[6] Similar results were obtained by both

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methods. Copolypeptides were found to contain a significantly higher mole fraction of (hydroxyethyl)-L-glutaminyl residues than that expected if hydroxyethanolamine and hydroxybutanolamine were equally reactive.

Circular dichroism measurements were conducted with solutions prepared by dilution of stock solutions with distilled deionized water. Concentrations were determined from a micro-Kjeldahl analysis of the stock solutions. A Jasco J-20 spectropolarimeter calibrated with *d*-10-camphorsulfonic acid[19] was employed for all circular dichroism measurements. In most cases the quartz cell used had a path length of 5 mm. Cell temperature was maintained with a circulating water bath connected to a brass water jacket surrounding the cell. Sample temperature was measured in the cell before and after each spectrum was recorded. The experimental helicity was calculated from [Θ] at 222 nm.

Calculations

Calculations were performed by using a sample of random copolypeptide chains generated with the aid of a pseudo random number generator. It is assumed that conformational properties of the polypeptide chains are dominated by nearest-neighbor interactions, aminolysis yields truly random copolypeptides, and end effects are treated in satisfactory approximation using the 2 × 2 version of the Zimm–Bragg[17] statistical weight matrix.

The statistical weight matrix for amino acid residue i is

$$\mathbf{U}_i = \begin{bmatrix} 1 & \sigma s \\ 1 & s \end{bmatrix}_i \tag{1}$$

where rows index the state of amino acid residue $i - 1$, columns index the state of amino acid residue i, and the order of indexing is c, h. An amino acid residue whose conformation is that found in the helix is in state h, and all other conformations are described by the state c. The statistical weight for propagation of a helical segment is s, and the statistical weight for initiation of a helical segment is σs. If there is compensation between long-range intramolecular interactions and solvent–macromolecule interactions, the configuration partition function, Z, for a partially helical copolypeptide containing n amino acid residues is

$$Z = \mathbf{J}^*\mathbf{U}_1\mathbf{U}_2 \ldots \mathbf{U}_n\mathbf{J} \tag{2}$$

where $\mathbf{J}^*$ = row (1, 0) and $\mathbf{J}$ = col (1, 1). The fraction f of residues that are helical is obtained as

$$f = n^{-1}Z^{-1}\mathbf{J}^*\mathbf{U}_1\mathbf{U}_2 \ldots \mathbf{U}_n\mathbf{J} \tag{3}$$

where $\mathbf{J}^*$ is now row (1, 0, 0, 0) and $\mathbf{J}$ is col (0, 0, 1, 1). The supermatrix $\hat{\mathbf{U}}_i$ is

$$\hat{\mathbf{U}}_i = \begin{bmatrix} \mathbf{U} & \mathbf{U}' \\ \mathbf{0} & \mathbf{U} \end{bmatrix}_i \tag{4}$$

where $\mathbf{0}$ denotes a null matrix and $\mathbf{U}_i'$ is obtained by zeroing all elements in the first column of $\mathbf{U}_i$.

A representative copolypeptide chain was grown as follows. A sequence of 300 random numbers, each between zero and one, was generated. The ith amino acid residue was assigned as (hydroxyethyl)-L-glutaminyl if the ith random number was less than or equal to the desired mole fraction of (hydroxyethyl)-L-glutaminyl residues in the copolypeptide. Otherwise the ith amino acid residue was assigned as (hydroxybutyl)-L-glutaminyl. A value for n of 300 was used because it gave a good approximation to the behavior of an infinitely long chain. The helical content of this chain was then calculated by eq 3. The desired helicity is the average of such f's over an infinitely large sample of random copolypeptides. For present purposes the helicity was approximated as the average of f's obtained from five independently grown sets, each containing 100 independently grown chains. This sample size was deemed sufficiently large because there was good agreement between averages deduced from the five independent sets.

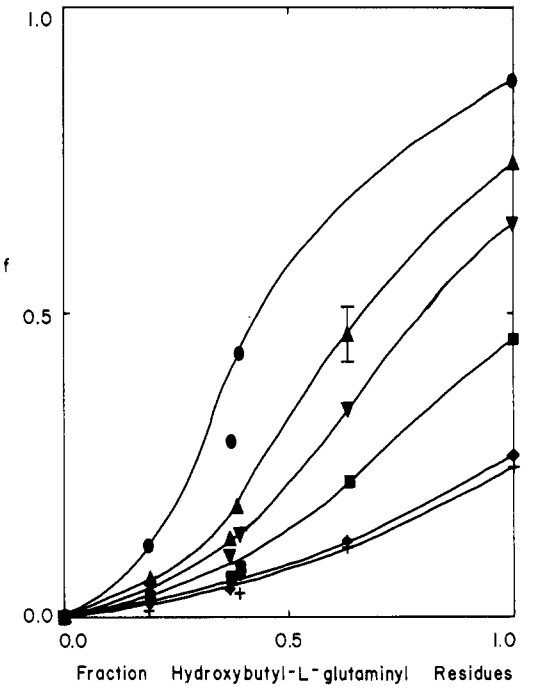

Figure 1. Experimentally determined helicities at temperatures of 4 (●), 25 (▲), 34 (▼), 45 (■), 56 (♦), and 64 (+) °C as a function of mole fraction of (hydroxybutyl)-L-glutaminyl residues. Solid lines are calculated from eq 3.

Values of σ and s for (hydroxybutyl)-L-glutaminyl residues were taken from von Dreele et al.[6] Those for (hydroxyethyl)-L-glutaminyl residues were obtained from the best fit to the experimentally determined helicities for the copolypeptides studied.

Results

Circular dichroism spectra had the shape expected for polypeptides undergoing a helix–coil transition. Figure 1 depicts helicities at several temperatures as a function of the mole fraction of (hydroxybutyl)-L-glutaminyl residues. Points are from experiment, and lines are the fit obtained from eq 3. Copolypeptides whose (hydroxybutyl)-L-glutaminyl mole fractions are 0.37 and 0.39 yield discordant circular dichroism spectra, particularly at the lower temperatures. The anomaly appears to reside in the copolypeptide that is 37% (hydroxybutyl)-L-glutaminyl because the other copolypeptide gives results that follow the trend established by the remaining copolypeptides and the two homopolypeptides. For this reason the copolypeptide with 37% (hydroxybutyl)-L-glutaminyl was ignored in the analysis used to obtain the σ and s reported here.

Figure 2 depicts the temperature dependence of s deduced for the (hydroxyethyl)-L-glutaminyl residue when its σ is taken to be 1×10^{-5}. This figure shows s to be less than unity throughout the temperature range covered, as expected from the nonhelical character of poly[(hydroxyethyl)-L-glutamine] in aqueous solution. An increase in temperature brings about a monotonic, nearly linear, decline in s. It decreases from 0.945 to 0.928 as the temperature rises from 4 to 64 °C. The relative error in s for the (hydroxyethyl)-L-glutaminyl residue is largest at the highest temperature because the copolypeptides have low

Table I
Parameters for Random Coil-to-Helix Transition in Water at 20 °C

parameter	L-glutaminyl[a]	(hydroxyethyl)-L-glutaminyl[b]	(hydroxypropyl)-L-glutaminyl[c]	(hydroxybutyl)-L-glutaminyl[c]
σ	3.3×10^{-5}	1×10^{-5}	2.2×10^{-4}	6.7×10^{-4}
ΔH, cal/mol	−493	−127	−168	−195
ΔG, cal/mol	13	35	13	−11
ΔS, cal/(mol K)	−1.7	−0.55	−0.616	−0.627

[a] From ref 22. [b] Present work. [c] From ref 6.

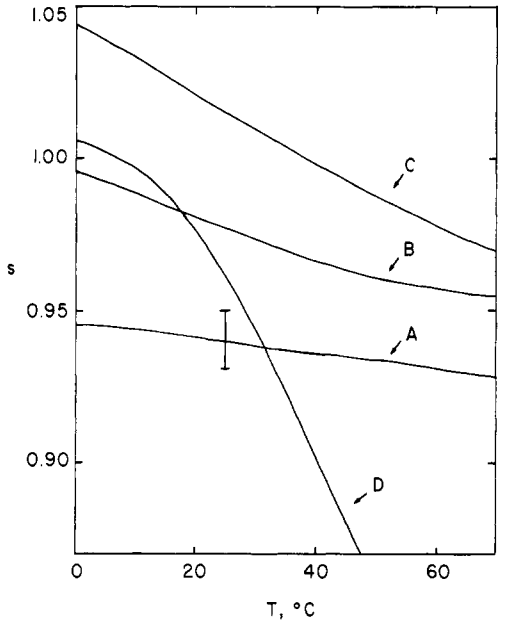

Figure 2. Temperature dependence of s for (hydroxyethyl)-L-glutaminyl (A), (hydroxypropyl)-L-glutaminyl (B), (hydroxybutyl)-L-glutaminyl (C), and L-glutaminyl (D). Curves B and C are from von Dreele et al.,[6] and curve D is from Denton et al.[22]

helical contents under these conditions. The relative error at 25 °C is indicated in Figure 2.

Discussion

The ability to theoretically describe experimentally determined helicities for the copolypeptides is only weakly dependent on the value assigned to σ for the (hydroxyethyl)-L-glutaminyl residue, as long as that value is small. Results presented here were obtained with $\sigma = 1 \times 10^{-5}$. Insensitivity of the calculation to σ for the (hydroxyethyl)-L-glutaminyl residue can be attributed to its being much smaller than σ for the host, (hydroxybutyl)-L-glutaminyl (Table I). Nearly all helix initiation in the copolypeptides therefore occurs at (hydroxybutyl)-L-glutaminyl residues, causing the value assigned to σ for the (hydroxyethyl)-L-glutaminyl residue to be of little consequence.

Our σ for the (hydroxyethyl)-L-glutaminyl residue in water is much smaller than the value of $(1.5\text{–}4.4) \times 10^{-3}$ estimated in water–2-propanol mixtures.[21] While our σ for (hydroxyethyl)-L-glutaminyl residues is smaller than those found by von Dreele et al.[6] for (hydroxypropyl)L-glutaminyl and (hydroxybutyl)L-glutaminyl residues in water (Table I), it follows qualitatively the trend established by these two residues. In this series helix formation in water becomes less cooperative as methylene groups are added to the periphery of the side chain. The L-glutaminyl residue itself has a much larger σ[22] and consequently does not behave as a member of the (hydroxyalkyl)-L-glutaminyl series.

In addition to our results for the (hydroxyethyl)-L-glutaminyl residue, Figure 2 also depicts s obtained by von

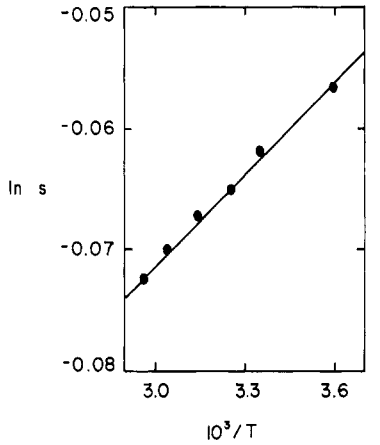

Figure 3. Linear relationship (correlation coefficient 0.996) between ln s and $1/T$ for (hydroxyethyl)-L-glutaminyl residues in water.

Dreele et al.[6] for (hydroxypropyl)-L-glutaminyl and (hydroxybutyl)-L-glutaminyl residues. (Hydroxyethyl)-L-glutaminyl has the smallest s, as expected. In all three cases s decreases as temperature increases. While the temperature effect is smallest with (hydroxyethyl)-L-glutaminyl, the form of the thermally induced change is similar for all three amino acid residues. The s for the L-glutaminyl residue, from Denton et al.,[22] also decreases with an increase in temperature. The form of its temperature dependence, however, is quite different from that seen with the (hydroxyalkyl)-L-glutaminyl residues. Specifically, d^2s/dT^2 is strongly negative at the lower end of the temperature range for L-glutaminyl, while it is zero or even slightly positive for (hydroxyalkyl)-L-glutaminyl residues. According to data presented in Figure 2, a hypothetical poly[(hydroxyalkyl)-L-glutamine] with a nonexistent "alkyl" should have an s in water that is less than 0.9 and nearly independent of temperature. The observed behavior of the L-glutaminyl residue is quite different. Clearly, the nature of the terminal group in the side chain plays a role in dictating the tendency for helix formation.

Figure 3 demonstrates that ln s for the (hydroxyethyl)-L-glutaminyl residue is a linear function of $1/T$, signifying that the enthalpy change for helix propagation is independent of temperature over the range covered. Thermodynamic parameters for helix propagation at 20 °C are collected in Table I along with values obtained by Scheraga and co-workers[6,22] for the L-glutaminyl, (hydroxypropyl)-L-glutaminyl, and (hydroxybutyl)-L-glutaminyl residues. In the (hydroxyalkyl)-L-glutaminyl series, the enthalpy change becomes more negative by about 34 cal/mol with the addition of a methylene group, while the free energy change at 20 °C becomes more negative by about 23 cal/mol. Extrapolation from the known behavior of the three poly[(hydroxyalkyl)-L-glutamines] leads to the prediction that the next higher member of the series, poly[(hydroxypentyl)-L-glutamine], would have σ near 10^{-3} and s greater than unity when the temperature is below 60 °C. At 25 °C the predicted helical content for an in-

finitely long chain is greater than 0.8.

Conformational energy calculations have been performed for the (hydroxyethyl)-L-glutaminyl residue with the objective of determining preferred conformations of the side chain.[23] The side chain was found to participate in intraresidue hydrogen bond formation between the terminal hydroxyl group and the carbonyl group in the peptide backbone. This hydrogen bond can be formed without appreciable alteration in the unperturbed dimensions of the polypeptide chain. When unperturbed dimensions are calculated by using a conformational energy surface that incorporates this hydrogen-bonding interaction,[23] they remain in agreement with the experimental result.[1] Thus unperturbed dimensions do not provide a sensitive test for the importance of intraresidue hydrogen bond formation by (hydroxyethyl)-L-glutaminyl in water.

Formation of an intraresidue hydrogen bond involving the carbonyl group in the polypeptide backbone should discourage helix formation. If this intraresidue hydrogen bond is assumed to be relatively inaccessible to higher (hydroxyalkyl)-L-glutaminyl residues, helix formation by (hydroxyethyl)-L-glutaminyl might be expected to be more difficult than that predicted from the observed behavior of (hydroxypropyl)-L-glutaminyl and (hydroxybutyl)-L-glutaminyl residues. Results reported in Figure 2 and Table I show that there are no anomalies in the behavior of s for the (hydroxyethyl)-L-glutaminyl residue. The intramolecular hydrogen-bonded conformations predicted for the (hydroxyethyl)-L-glutaminyl residue apparently are of little consequence in a hydrogen-bonding solvent such as water.

Acknowledgment. This research was supported by National Science Foundation Grant PCM 78-22916. We thank Professor E. W. Blakeney for performing the ninhydrin analysis.

Registry No. Poly(γ-benzyl L-glutamate), 25014-27-1; (*S*)-poly[imino[1-oxo-2-[3-oxo-3-(phenylmethoxy)propyl]-1,2-ethanediyl]], 25038-53-3; ethanolamine, 141-43-5; 4-butanolamine, 13325-10-5.

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