form of the segment distribution, which will be changing markedly as the solvent composition is varied near the theta state, plays a more dominant role than the radius of gyration in determining the friction properties. As the molecule passes into the subtheta state, it will form a tighter coil, and the increased segment density and preferential adsorption at low molecular weights may result in the solvent being trapped in the molecule and being dragged along with it. This change in the draining property of the molecule may increase its hydrodynamic size.

The values of k_D, the slopes of the D–c plots normalized against D_0, can be used to indicate the theta-state composition for the mixed solvent. Thermodynamic arguments[8] relate k_D to k_f, the concentration coefficient for the friction coefficient

$f\,[= f_0(1 + k_f c + \ldots)]$:

$$k_D + k_f = 2A_2M - \bar{v}$$

where $\bar{v}$ is the partial specific volume of the polymer (=0.92 mL g^{-1} for polystyrene). Several theories exist which predict values for k_f. For example, Yamakawa[10] proposes that $k_f = \lambda(x)A_2M + \bar{v}_h$, where $\lambda(x)$ is a function depending on x $(=z/\alpha^3)$ and equals 1.345 for $x = 0$, the theta state, and $\bar{v}_h$ is the specific hydrodynamic volume of the polymer, which can be obtained from D_0. Pyun and Fixman[11] have considered two models; the hard-sphere model, where the spheres are impenetrable, gives a value of $k_f = 7.16\bar{v}_h$, while for the soft-sphere model, where the molecules are interpenetrable, $k_f = 2.23\bar{v}_h$ at the theta state, rising to the hard-sphere value as z increases.

In this work, A_2M is unknown, but should be zero at the theta state. Hence under this condition

$$\begin{aligned} k_D{}^\theta &= -\bar{v}_h - \bar{v} \qquad \text{(ref 10)} \\ &= -7.16\bar{v}_h - \bar{v} \text{ (hard sphere; ref 11)} \\ &= -2.23\bar{v}_h - \bar{v} \text{ (soft sphere; ref 11)} \end{aligned}$$

Thus $k_D{}^\theta$ should vary as $\bar{v}_h$ since $\bar{v}_h \gg \bar{v}$, and thus as $M^{1/2}$.

We have plotted log $(-k_D)$ against log $\bar{M}_w$ for the three solvent mixtures in Figure 7, and the composition $\phi = 0.8025$ gives a slope 0.45 which is closest to 0.5 of the three slopes. This adds support to the strong evidence from the variation of log D_0 with log $\bar{M}_w$, which suggests that this value of ϕ represents the theta composition. For this composition, the line fitted to the points in Figure 7 would point to a value of about 7 for the coefficient of $\bar{v}_h$ in the expression for $k_D{}^\theta$, and thus favors the hard-sphere model of Pyun and Fixman. However, this result can only be taken as an indication.

In conclusion, we would emphasize that the results of these diffusion measurements of high precision do not reflect the effects observed in the viscometry measurements of Dondos and Benoit on the same system, which they attributed to the preferential adsorption. Indeed, our measurements would indicate a well-defined diffusion theta composition at $\phi = 0.8025$. However, the z-parameter theory is inadequate to explain the curious behavior of the hydrodynamic radius with polymer molecular weight and solvent composition.

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Hydrodynamic Properties and Unperturbed Dimensions of Poly(γ-hydroxy-L-proline) in Aqueous Solution

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ABSTRACT: Intrinsic viscosities and sedimentation coefficients have been measured as a function of molecular weight for poly(γ-hydroxy-L-proline) in water. The molecular weight range covered is 9 000–35 000. High molecular weight poly(γ-hydroxy-L-proline) in water has the hydrodynamic behavior of a random coil immersed in a good solvent, as judged by d ln $[\eta]$/d ln M, d ln s_0/d ln M, dβ/dM, and the size of β $[= Ns_0[\eta]^{1/3}\eta M^{-2/3}(1 - \bar{v}\rho)^{-1}]$. The characteristic ratio, $(\langle r^2\rangle_0/n_p l_p^2)_\infty$, is found to be 15.9 ± 1.6, which is not significantly different from the result of 13.7 ± 0.9 obtained for poly(L-proline) by Mattice and Mandelkern under the same conditions. This observation casts doubt on the importance of intrachain hydrogen bonded bridges from the hydroxyl group to a carbonyl oxygen via a water molecule. The characteristic ratios and the effects of calcium chloride on the hydrodynamic properties suggest that poly(L-proline) and poly(γ-hydroxy-L-proline) possess a similar source of flexibility which is often overlooked. Two possibilities for this flexibility are a second energy minimum about the C^α–C' bond and cis-trans isomerization about the peptide bond.

The pyrrolidine ring exerts a profound effect on the conformational properties of poly(L-proline) and poly(γ-hydroxy-L-proline). It confines the dihedral angle ϕ[1] to the vicinity of 120°, and steric interactions between neighboring pyrrolidine rings also impose severe restrictions on the accessible values for ψ. For these reasons the two homopolypeptides share certain conformational properties. The most detailed x-ray diffraction patterns for poly(γ-hydroxy-L-

Table I
Measured Properties of the Poly(γ-hydroxy-L-proline) Samples in Water

Sample	$10^{-3}M_n$	$10^{-3}M_w$	M_w/M_n	$10^{13}s_0$[a]	$[\eta]$[b]	10^3d ln $[\eta]$/dT[c]	10^4A_2[d,e]	10^4A_2[d,f]
HP15		8.9 ± 0.6		1.08 ± 0.10	0.29	−2.6		10
F3	15.6 ± 1.3	16.5 ± 0.9	1.06 ± 0.14	1.12 ± 0.03	0.63	−4.7	12	13
F2	20.4 ± 1.8	20.7 ± 1.2	1.01 ± 0.14	1.28 ± 0.06	0.82	−4.3	2	5
F1	35.0 ± 3.0	35.2 ± 3.0	1.01 ± 0.18	1.48 ± 0.07	1.08	−4.8	5	10

[a] At 20 °C, s^{-1}. [b] At 30 °C, dL/g. [c] Over the temperature range 5–55 °C, deg^{-1}. [d] There is a large uncertainty associated with the second virial coefficients (see text). Units are cm^3 mol/g^2. [e] From eq 1 and the straight lines in Figure 1. [f] From eq 2 and the straight lines in Figure 2.

proline) are obtained in the state known as form A.[2] Under these conditions the peptide units adopt the trans conformation and the polypeptide chain forms a left-handed helix of dimensions virtually identical to that found in poly(L-proline) form II.[3-5] The packing of the helices is altered in poly(γ-hydroxy-L-proline) form A to permit the formation of intermolecular hydrogen bonds between the hydroxyl groups and carbonyl groups.[2] A structure has not been convincingly assigned to another solid state modification, poly(γ-hydroxy-L-proline) form B, because its x-ray diffraction pattern exhibits only a few diffuse reflections.[6] The vacuum ultraviolet absorption spectrum of an oriented poly(γ-hydroxy-L-proline) film, however, has been interpreted[7] as evidence that the chain conformation in form B is related to that found in poly(L-proline) form I.[8] The peptide units adopt the cis conformation in poly(L-proline) form I, and a right-handed helix is formed.

Substantial differences are observed in several of the solution properties of poly(L-proline) and poly(γ-hydroxy-L-proline). Poly(γ-hydroxy-L-proline) is readily soluble in water, but it is insoluble in most organic solvents.[9] In contrast, poly(L-proline) is readily soluble in several organic solvents and dissolves in water only upon cooling.[10-12] Poly(L-proline), but not poly(γ-hydroxy-L-proline), precipitates from an aqueous solution upon heating.[10,13-15] Optical activity measurements demonstrate that a reversible, cooperative isomerization about the peptide bonds in poly(L-proline) can be achieved by appropriate adjustments in solvent composition.[16-18] A comparable phenomenon has not been observed with poly(γ-hydroxy-L-proline). Reversible cooperative isomerization is observed if the hydroxyl groups are modified, as in poly(*O*-acetyl-γ-hydroxy-L-proline)[9,19] or poly(*O*-benzyl-γ-hydroxy-L-proline).[19]

The hydrodynamic behavior of poly(L-proline) is known as a function of molecular weight in several solvents.[20] These measurements have been used to estimate the characteristic ratio, $\langle r^2\rangle_0/n_p l_p^2$, where $\langle r^2\rangle_0$ is the unperturbed mean square end-to-end distance for a polypeptide containing n_p virtual bonds[21] of length l_p. Equivalent information has not been reported for poly(γ-hydroxy-L-proline) because available samples are all of low molecular weight. Fractionation of a commercially supplied preparation of poly(γ-hydroxy-L-proline) has provided us with nearly monodisperse samples whose molecular weights range up to 35 000. The present objective is to report the results of our study of these poly(γ-hydroxy-L-proline) fractions in water and to assess the implications for the conformational effects arising from the substitution of a γ-hydroxy-L-prolyl residue for an L-prolyl residue in a polypeptide chain.

Experimental Section

Materials. Two poly(γ-hydroxy-L-proline) samples were used. The lower molecular weight sample, designated HP15, was used as supplied by Miles Laboratories. Gel permeation chromatography of the higher molecular weight sample (Sigma Chemical Co.) using a 5 × 80 cm Sephadex G-100 column produced three fractions, designated F1, F2, and F3. Calcium chloride solutions were prepared by dilution of a saturated stock solution.

Osmometry. Osmotic pressures were measured at 30 °C in water using a Mechrolab 501 high speed membrane osmometer equipped with an S & S B-20 membrane.

Sedimentation. Sedimentation equilibrium measurements were conducted at 25 °C and sedimentation velocity measurements were conducted at 20 °C using a Beckman Model E analytical ultracentrifuge. Interference optics were used at equilibrium and Schlieren optics were used for velocity measurements. The partial specific volume of poly(γ-hydroxy-L-proline) in water is 0.655 ± 0.010 cm^3/g.[19]

Viscosities. Flow times were measured in water at 5, 30, and 55 °C and in aqueous calcium chloride at 30 °C, using Cannon-Ubbelohde semimicro dilution viscometers.

Results

Molecular Weights. Number average molecular weights, M_n, for the fractions were obtained by application of eq 1 to the reduced osmotic pressures shown in Figure 1.

$$\pi(cRT)^{-1} = M_n^{-1} + A_2c \qquad (1)$$

The osmotic pressure is π, c is the poly(γ-hydroxy-L-proline) concentration, R is the gas constant, T is the temperature, and A_2 is the second virial coefficient. Molecular weights are presented in Table I. Osmotic pressures were not measured for sample HP15 because of the anticipated difficulty with permeation of solute through the membrane for an unfractioned polymer of low weight average molecular weight.

Weight average molecular weights, M_w, were obtained in the manner employed previously for poly(L-proline).[20] An apparent weight average molecular weight, M_w^{app}, was evaluated from the slope of the logarithm of the fringe number vs. the square of the displacement from the axis of rotation.[22] The true molecular weight was obtained by extrapolation to zero concentration according to eq 2.

$$(M_w^{app})^{1/2} = M_w^{-1/2} + A_2M_w^{1/2}(c_t + c_b)/2 \qquad (2)$$

Equilibrium concentrations at the top and bottom of the cell are denoted by c_t and c_b, respectively. The results obtained from the intercepts in Figure 2 are shown in Table I. The fractions obtained by the Sephadex treatment have M_w/M_n near unity and consequently possess a narrow molecular weight distribution.

Intrinsic Viscosities. Intrinsic viscosities were obtained in water at 5, 30, and 55 °C. The results at 30 °C are collected in Table I. Temperature coefficients for the intrinsic viscosities are large and negative, as shown by Figure 3 and Table I. The limiting temperature coefficient, obtained as the intercept of d ln $[\eta]$/dT vs. $1/M_w$, is −0.005 deg^{-1}. Equivalent treatment of previously reported[20,23] data for poly(L-proline) also yields a large negative temperature coefficient, −0.008 deg^{-1}. In this respect poly(L-proline) and poly(γ-hydroxy-L-proline) are reminiscent of various derivatives of cellulose.[24]

Figure 4 presents the molecular weight dependence of the intrinsic viscosities obtained at 30 °C in water. This figure also

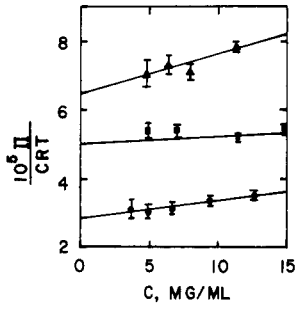

Figure 1. Reduced osmotic pressures for poly(γ-hydroxy-L-proline) samples F1 (circles), F2 (squares), and F3 (triangles) in water at 30 °C.

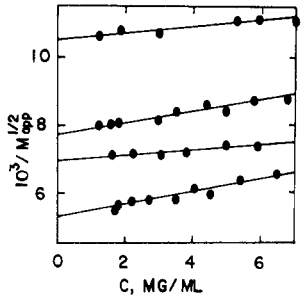

Figure 2. Concentration dependence of the apparent weight average molecular weights measured by equilibrium ultracentrifugation in water at 25 °C. The concentration is the average of the equilibrium concentrations at the top and bottom of the cell. The poly(γ-hydroxy-L-proline) samples are HP15, F3, F2, and F1, reading from top to bottom.

displays previously reported[20,23] intrinsic viscosities obtained for poly(L-proline) samples in water at 30 °C. It is apparent that a common line provides a reasonable fit to the results obtained with both polypeptides. The nature of the molecular weight dependence of the temperature coefficients is such that the curvature apparent in Figure 4 will diminish as temperature increases. The best straight line through the points representing poly(γ-hydroxy-L-proline) samples F1, F2, and F3 has a slope of 0.68, which is well within the range anticipated for a random coil immersed in a good solvent.[25]

The intrinsic viscosities experience a marked reduction in the presence of calcium chloride. With fraction F1 the intrinsic viscosity in 5.7 M calcium chloride is only 1/6 of the result obtained in water. A smaller reduction, by a factor of 3, was obtained with a poly(γ-hydroxy-L-proline) sample with M_w = 9000.[26] The intrinsic viscosity of poly(L-proline) also undergoes a drastic reduction in aqueous calcium chloride.[20,23]

Sedimentation Coefficients. Sedimentation coefficients, s, for the poly(γ-hydroxy-L-proline) samples were measured at various initial concentrations. Extrapolation to the infinite dilution sedimentation coefficient, s_0, was accomplished using eq 3.

$$s^{-1} = s_0^{-1} + kc \quad (3)$$

The extrapolations are shown in Figure 5, and the resulting sedimentation coefficients are collected in Table I.

Figure 6 shows the manner in which the sedimentation coefficients depend on molecular weight. Curvature is apparent, as was the case for the equivalent representation of the intrinsic viscosities. The best straight line through the data for poly(γ-hydroxy-L-proline) samples F1, F2, and F3 has a slope of about 0.38, suggesting that the polymer behaves as a random coil in a good solvent.[25] An appreciable uncertainty must be assigned to the slope due to the uncertainties in the

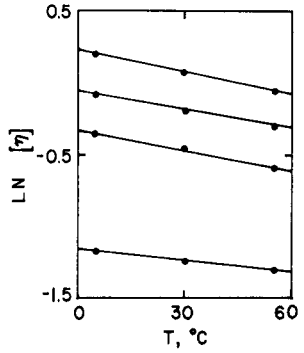

Figure 3. Temperature dependence of the intrinsic viscosity of poly(γ-hydroxy-L-proline) in water. The samples are F1, F2, F3, and HP15, reading from top to bottom.

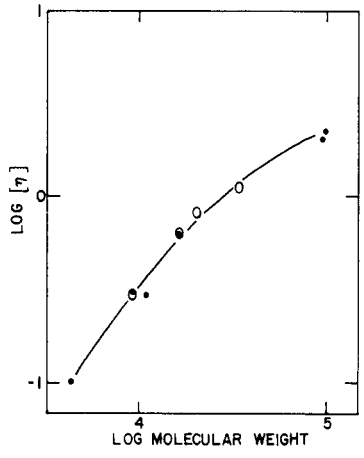

Figure 4. Molecular weight dependence of the intrinsic viscosity of poly(γ-hydroxy-L-proline) (open circles) and poly(L-proline) (small filled circles) in water at 30 °C. Results for poly(L-proline) are from ref 20 and 23.

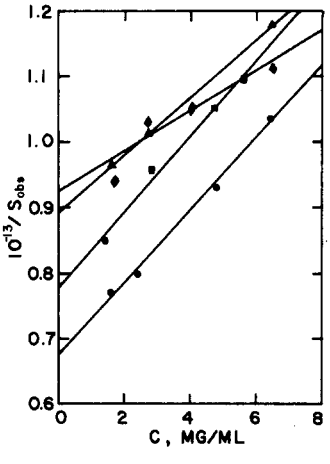

Figure 5. Concentration dependence of the sedimentation coefficients for poly(γ-hydroxy-L-proline) in water at 20 °C. The samples are F1, F2, F3, and HP15, reading from top to bottom.

sedimentation coefficients and the narrow range of molecular weight covered by the fractions studied.

A poly(L-proline) sample with an estimated molecular weight of 18 000–19 000 has been reported[11] to have an $s_{20,w}$ of 0.83 × 10^{-13} s. This result is about 70% as large as the sedimentation coefficient estimated from Table I for a poly(γ-hydroxy-L-proline) sample of the same molecular weight. The buoyancy factor, $1 - \bar{v}\rho$, for poly(L-proline) in water is only about 70% of the value for poly(γ-hydroxy-L-proline), ac-

Table II
Parameters Calculated from the Experimental Results for Poly(γ-hydroxy-L-proline)

Sample	$10^{-6}\beta^a$	$10^7 D_0^{a,b}$	$10^8 f_0^{a,c}$	α		$\langle r^2\rangle_0/n_p l_p^2$		$\langle r^2\rangle_0^{1/2}/3.12n_p$	
				SE[d]	OP[e]	SE[d]	OP[e]	SE[d]	OP[e]
HP15	2.93 ± 0.37	8.6 ± 1.1	4.7 ± 0.6	1.09		8.6		0.40	
F3	2.62 ± 0.24	4.8 ± 0.4	8.4 ± 0.8	1.10	1.09	11.6	11.8	0.34	0.36
F2	2.82 ± 0.32	4.3 ± 0.5	9.3 ± 1.1	1.04	1.02	12.5	14.7	0.32	0.35
F1	2.51 ± 0.33	3.0 ± 0.4	13.6 ± 1.8	1.10	1.05	12.9	13.9	0.25	0.26

[a] At 20 °C. [b] Diffusion coefficient, cm^2/s. [c] Frictional coefficient, g/s. [d] Using the A_2 and M_w obtained from sedimentation equilibrium. [e] Using the A_2 and M_n obtained from osmotic pressure.

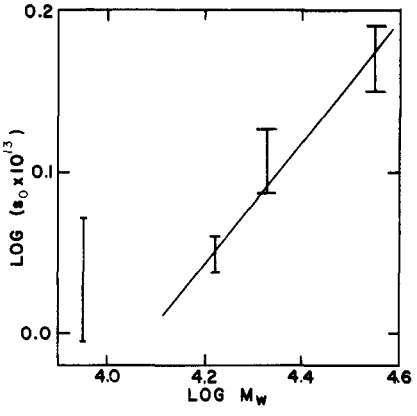

Figure 6. Molecular weight dependence of the sedimentation coefficients for poly(γ-hydroxy-L-proline) samples F1, F2, F3, and HP15 in water at 20 °C.

counting for the difference in the sedimentation coefficients of the two polypeptides.

Second Virial Coefficients. The data represented in Figures 1 and 2 are sufficiently precise to provide accurate intercepts and molecular weights. A considerably higher uncertainty exists in the slopes, and for this reason there is a large uncertainty in the estimation of the second virial coefficients. The numbers presented in Table I are obtained from the straight lines in Figures 1 and 2. Second virial coefficients are positive and tend to be large, on the order of 10^{-3} cm^3 mol/g^2. Large second virial coefficients are also obtained for poly(L-proline) in water.[20]

Discussion

Molecular Weight Dependence of the Intrinsic Viscosity and Sedimentation Coefficient. The best values of d ln $[\eta]$/d ln M and d ln s_0/d ln M for the three poly(γ-hydroxy-L-proline) fractions are 0.68 and 0.38, respectively. These results compare favorably with 0.5–0.8 and 0.4–0.5, the ranges anticipated for random coil polymers immersed in a good solvent.[25] For rigid helices d ln $[\eta]$/d ln M = 1.8 and d ln s_0/d ln M = 0.2,[25,27] results which are clearly incompatible with the data obtained using the three poly(γ-hydroxy-L-proline) fractions.

Curvature is apparent at low molecular weight in the ln $[\eta]$ vs. ln M plots obtained for several random coil polymers in good solvents.[28] A similar effect is apparent with poly(γ-hydroxy-L-proline) in water. The nature of the curvature in ln s_0 vs. ln M at low molecular weight is consistent with the curvature in ln $[\eta]$ vs. ln M.

Combination of Sedimentation Coefficient and Intrinsic Viscosity. A further test of the validity of the conclusions obtained from d ln $[\eta]$/d ln M and d ln s_0/d ln M is provided by combination of the sedimentation coefficient, intrinsic viscosity, and molecular weight according to eq 4.[29,30]

$$\beta = Ns_0[\eta]^{1/3}\eta M^{-2/3}(1 - \bar{v}\rho)^{-1} \quad (4)$$

Avogadro's number, the viscosity and density of the solvent, and the partial specific volume of the solute are denoted by N, η, ρ, and $\bar{v}$, respectively. The β are collected in Table II and presented graphically in Figure 7. The uncertainty in β arises from the uncertainties in s_0, M, and $\bar{v}$. There is a slight trend for the β to increase at low molecular weight. However, the results for the three fractions are consistent with a molecular weight independent β of (2.5–2.8) × 10^6. Values of β for six different polymer–solvent systems (polystyrene–methyl ethyl ketone, polystyrene–toluene, cellulose acetate–acetone, polysarcosine–water, polyisobutylene–cyclohexane, and poly(methyl methacrylate)–acetone)[25] are independent of molecular weight and in the range (2.3–2.7) × 10^6. Consequently the β obtained with the poly(γ-hydroxy-L-proline) fractions exhibit the behavior anticipated for a random coil polymer. This conclusion is in harmony with that obtained from d ln $[\eta]$/d ln M and d ln s_0/d ln M.

It is of interest to compare the experimental β with those which should be obtained if the form A chain geometry were rigorously maintained in solution. The length of the helix would then be 3.12 Å per residue.[2] The interchain spacing for form A in the solid state is close to 7.5 Å.[2] In solution the effective diameter might be somewhat larger, due to close packing of the pyrrolidine rings in the solid state. Therefore we shall consider the diameter to lie within the range 7.5–10 Å. The calculated β are obtained from these dimensions and the tables reported in ref 30.

The predicted β for sample HP15, the low molecular weight sample, are 2.81 and 2.72 × 10^6 for 7.5 and 10 Å diameters, respectively. Either value is consistent with the experimental result of (2.93 ± 0.37) × 10^6. The β calculated for the solid state geometry increase with molecular weight, an effect which is not observed in the experimental β. For the highest molecular weight sample, F1, the calculated values for form A are 3.30 and 3.21 × 10^6 (for diameters of 7.5 and 10 Å, respectively). These β are much larger than the result obtained experimentally with F1.

The hydrodynamic properties of the poly(γ-hydroxy-L-proline) fractions can be summarized as follows: d ln $[\eta]$/d ln M, d ln s_0/d ln M, dβ/dM, and the size of β all behave in the manner found with random coil polymers in good solvents. They are in conflict with the behavior which would be found if the form A chain configuration were rigorously maintained in solution.

Unperturbed Dimensions. The characteristic ratio for poly(γ-hydroxy-L-proline) can be calculated from the data assembled in Table I using eq 5–7,[31–33] as has been done previously using data obtained with other random coil polypeptides in good solvents.[20,33–37]

$$[\eta]_\theta = [\eta]/\alpha^3 \quad (5)$$

$$A_2M/[\eta] = 2^{5/2}\pi N(27\Phi)^{-1} \ln [1 + \pi^{1/2}(\alpha^2 - 1)/2] \quad (6)$$

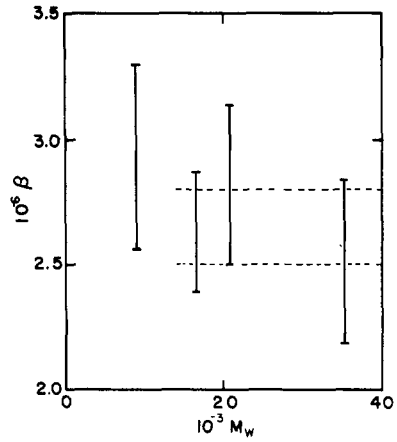

Figure 7. Molecular weight dependence of β for poly(γ-hydroxy-L-proline) in water at 20 °C.

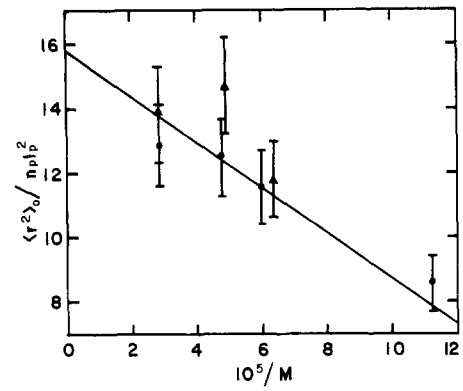

Figure 8. Molecular weight dependence of the characteristic ratio of poly(γ-hydroxy-L-proline) in water. Circles denote the results obtained using M_w and the A_2 obtained from sedimentation equilibrium. Triangles denote results obtained using M_n and the A_2 obtained using osmometry.

$$\langle r^2\rangle_0/n_p l_p^2 = ([\eta]_\theta/\Phi M^{1/2})^{2/3} M_0/l_p^2 \quad (7)$$

The intrinsic viscosity under unperturbed conditions is $[\eta]_\theta$, α is the expansion coefficient, M_0 is the amino acid residue weight, and Φ is a constant. The value assigned to Φ for polymers in good solvents is 0.0021, with distances expressed in ångström units and the intrinsic viscosity in dL/g. No consideration need be given to polydispersity for poly(γ-hydroxy-L-proline) samples F1, F2, and F3 because their molecular weight distributions are narrow. In the absence of equivalent information for sample HP15, we shall treat it as though it were monodisperse.

The expansion coefficients obtained from eq 6 are shown in Table II. They are slightly greater than unity. Fortunately, the large uncertainty in the second virial coefficient does not produce a similar uncertainty in α. Characteristic ratios are shown in Table II and in Figure 8. A significantly lower characteristic ratio is obtained for the sample of lowest molecular weight. Extrapolation of $\langle r^2\rangle_0/n_p l_p^2$ vs. $1/M$ to $1/M = 0$ yields $(\langle r^2\rangle_0/n_p l_p^2)_\infty = 15.8$ with an uncertainty of about 10%. While the characteristic ratio of poly(γ-hydroxy-L-proline) might be slightly larger than 13.7 ± 0.9, the result obtained with poly(L-proline),[20] the difference does not exceed the experimental uncertainty in the measurements. This result provides confirmation for the prediction by Schimmel and Flory[38] that poly(L-proline) and poly(γ-hydroxy-L-proline) should have equivalent unperturbed dimensions. The characteristic ratios for poly(γ-hydroxy-L-proline) and poly(L-proline) exceed those obtained for homopolypeptides bearing a CH_2R side chain in the L configuration,[33,37] for various copolypeptides,[34,36] and for denatured proteins.[35]

A means of representing the deviation of the poly(γ-hydroxy-L-proline) chain conformation in solution from that adopted by form A in the solid state[2] is provided by the ratio of $\langle r^2\rangle_0^{1/2}$ to the end-to-end distance for a form A helix. The values of this ratio are collected in Table II. All samples studied have a root-mean-square end-to-end distance which is less than half the value characteristic of the form A helix. This ratio decreases to ¼ for F1, the sample with a molecular weight of 35 000. A poly(γ-hydroxy-L-proline) chain of this molecular weight exhibits extensive coiling in solution.

While the $(\langle r^2\rangle/n_p l_p^2)_\infty$ for poly(γ-hydroxy-L-proline) and poly(L-proline) are larger than those reported for other polypeptides,[33-37] they are nonetheless substantially smaller than those calculated from many conformational energy maps reported for poly(L-proline) and poly(γ-hydroxy-L-proline).[20,38,39] This discrepancy signifies that poly(L-proline) and poly(γ-hydroxy-L-proline) possess a source of flexibility which is often overlooked.

Proton NMR measurements in aqueous solution demonstrate that the pyrrolidine ring in poly(L-proline) interconverts rapidly between two puckered conformations,[40] while a single conformation is favored in poly(γ-hydroxy-L-proline).[41] Pulse Fourier transform ^{13}C NMR shows rapid ring motion in both homopolypeptides, with the pyrrolidine ring in poly(L-proline) being the more mobile.[42] These observations have a direct bearing on the unperturbed dimensions because the severity of the steric interaction between adjacent pyrrolidine rings, and hence the potential function for rotation about the C′–C$^\alpha$ bond, depend on the conformation adopted by the pyrrolidine rings.[43,44] The most important consequence of a simple treatment of flexibility in the pyrrolidine ring is the appearance of a second energy minimum which is located about 180° from the customary minimum.[43,44] Although populated to only a minor extent, this minimum brings about a drastic reduction in the unperturbed dimensions. The simple provision for pyrrolidine ring flexibility[43,44] readily produces characteristic ratios compatible with those determined experimentally. A recent more extensive investigation of the consequences of pyrrolidine ring flexibility also finds a second minimum about the C′–C$^\alpha$ bond in Pro-Pro.[45] In contrast to the earlier work,[43,44] Madison[45] found that the preferred pyrrolidine ring conformations have puckering at C$^\gamma$, a result which is in harmony with solid state[4] and solution[40] measurements.

An alternative source of the added flexibility demanded by the characteristic ratios is rotation about the peptide bond. The 270 MHz proton Fourier transform NMR spectra of poly(L-proline) in D_2O exhibit a weak resonance which indicates that 2–3% of the peptide bonds adopt the cis conformation.[46] The presence of a small number of cis peptide bonds in a poly(L-proline) chain has an important effect on the unperturbed dimensions and can lead to the computation of characteristic ratios in the range found experimentally.[47]

It is tempting to assert that the source of the added flexibility should be the same for both homopolypeptides because of the similarity in their covalent structures and characteristic ratios. The added flexibility might be achieved by freer rotation about the C′–C$^\alpha$ bond, isomerization about the peptide bond, or a combination of both processes. There is no compelling evidence for the existence of cis peptide bonds in poly(γ-hydroxy-L-proline), either in the solid state or in solution. It is, of course, possible that a few cis peptide bonds are present in poly(γ-hydroxy-L-proline) when dissolved in water and that they have simply escaped detection. Indeed, the spectral region anticipated for the proton NMR resonance diagnostic for a cis peptide bond is obscured by the C$^\gamma$–H resonance in aqueous poly(γ-hydroxy-L-proline).[48]

Effect of Calcium Chloride. Previous work[26] has shown that calcium chloride reduces the intrinsic viscosity and eliminates the positive 225-nm circular dichroism band in a poly(γ-hydroxy-L-proline) sample with M_w = 9000. Similar effects occur with poly(L-proline), although at somewhat lower salt concentrations than those required for poly(γ-hydroxy-L-proline).[26] Results reported here for fraction F1 show that, as with poly(L-proline),[20,23] ln [η] for poly(γ-hydroxy-L-proline) becomes more sensitive to calcium chloride as the molecular weight increases. As a consequence d ln [η]/d ln M for both polypeptides decreases in size as the calcium chloride concentration increases. Proton and ^{13}C NMR studies demonstrate that the calcium chloride induced collapse in the dimensions of poly(L-proline) arises from a salt-induced isomerization about randomly located peptide bonds.[49,50] Since calcium chloride has similar effects on the two polypeptides, the poly(γ-hydroxy-L-proline) chain might be expected to exist with an appreciable number of its peptide bonds in the cis conformation. While the ^{13}C NMR spectrum of poly(γ-hydroxy-L-proline) in 6 M lithium bromide has been reported to show the existence of only one dominant isomer,[51] unpublished spectra reveal the presence of cis peptide bonds at 70 °C in 4 M calcium chloride.[52]

Implications for the Stability of the Collagen Helix. Recent work on synthetic sequential copolypeptides[53] and modified collagens[54-56] has shown that the stability of the collagen helix increases upon the substitution of γ-hydroxy-L-proline for L-proline. The stereochemistry of the attachment of the hydroxyl group to the C^γ atom also affects the stability of the helix.[57] One explanation offered for the hydroxyproline effect is the formation of an intrachain hydrogen bonded bridge from the hydroxyl group to a carbonyl oxygen atom via a water molecule.[58,59] If such hydrogen bonds exist in aqueous collagen, they should be formed equally well in aqueous poly(γ-hydroxy-L-proline) when the polypeptide chain adopts a conformation similar to the chain conformation found in collagen. These hydrogen bonds would be expected to stabilize the extended collagen-like conformation of poly(γ-hydroxy-L-proline), while the lack of a hydroxyl group would prohibit a similar stabilization for this conformation in poly(L-proline). Consequently the hydrogen bonds should cause the dimensions of poly(γ-hydroxy-L-proline) to exceed those of poly(L-proline). The observation of virtually identical characteristic ratios for poly(γ-hydroxy-L-proline) and poly(L-proline) demonstrates that these intrachain hydrogen bonded bridges, if present, are not strong enough to produce significant effects on the chain conformation. It is more likely that the hydroxyproline effect on the stability of the collagen helix has either an interchain origin[57] or is a consequence of alterations in the flexibility of the pyrrolidine ring.[44]

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