MAn and lecithin micelle is thought to be weakened by the disorder of lecithin micelle caused by an increase in temperature.

Conclusion

The copolymerization of IN with MAn gave an alternating copolymer in the presence and absence of lecithin. The chiral surface active (amphiphilic) substance, lecithin, lowered the rate of copolymerization and intrinsic viscosity of the copolymer. The influences of lecithin on the copolymerization are considered to be due to an electrostatic interaction between MAn and the polar head group of lecithin. In nonpolar solvents such as benzene, lecithin molecules associate to form reversed micelles. The nonpolar monomer, IN, may exist at the exterior of the reversed lecithin micelle, while the polar monomer, MAn, may be captured within the micelles, in which an electrostatic interaction between MAn and lecithin can be present. The asymmetric induction copolymerization of IN with MAn is considered to be caused by the electrostatic interaction between MAn and the polar head group of the chiral amphiphilic substance, lecithin. The copolymerization may proceed via CT complex of IN and MAn affected by the lecithin micelle. The asymmetry is thought to be induced at the site of the MAn monomer units in the backbone of the copolymer. The λ_0 values of the copolymers supported this assumption.

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Notes

Observation of Cis Residues in Poly(γ-hydroxy-L-proline) in Aqueous Solution

JAMES J. DECHTER, DONALD S. CLARK, AND LEO MANDELKERN*

Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306.

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It has recently been established, by means of high-field (270 MHz) proton Fourier-transform NMR, that poly(L-proline) in D_2O at room temperature has about 2–3% of its imide bonds in the cis configuration.[1] As Schimmel and Flory[2] pointed out and as has been further discussed more recently[3] the presence of only a very small fraction of cis residues will markedly decrease the chain dimensions and average properties associated with the molecule and thus requires a reexamination of the published conformational energy maps. In a recent comprehensive report it has been shown that the average dimensional properties of poly(γ-hydroxyl-L-proline)[4] are very similar to those of poly(L-proline). The characteristic ratios of the two polymers in H_2O at 30 °C are 15.9 ± 1.6[4] and 13.7 ± 0.9,[5] respectively.

With the statistical conformation of the two polymers now shown to be similar, by experiment, the question as to whether cis imide groups are also present in poly(γ-hydroxyl-L-proline) becomes a matter of obvious importance. Unfortunately, proton NMR is not suitable for this purpose since the spectral region anticipated for the cis imide bond is obscured by the C^{γ}–H resonance in aqueous solution.[6] However, the utilization of ^{13}C NMR has shown potential for this purpose, despite the disadvantage of requiring much larger quantities of material. It has been successful in detecting cis peptide bonds in the copolymers poly(L-prolylglycyl) and poly(γ-hydroxyl-L-prolylglycyl).[7]

In the present work we wish to report the detection of a small percentage of cis imide bonds in poly(γ-hydroxyl-L-proline) in D_2O using high-field (67.9 MHz) ^{13}C Fourier-transform NMR. To establish the validity of the method we have also examined the corresponding spectrum of poly(L-proline) in D_2O at 30 °C. The pertinent region of the spectrum of this polymer is given in Figure 1. The delay times used to obtain the spectra were chosen in accord with the spin–lattice relaxation times, T_1, reported by Torchia and Lyerla.[7] Thus saturation of resonances is avoided. The assignment of the resonances is based on the previous work by Dorman et al.[8] The resonances assigned to the C^{γ} and C^{β} in the cis configuration can be clearly observed. Two small resonances appear downfield from the C^{β} trans. The resonance furthest downfield (ca. 30.6 ppm) is assigned to the cis C^{β} in accord with previous results for poly(L-proline). The other minor resonance that is observed cannot be definitely assigned but may be due to sequential effects of the cis isomer or another trans conformer. The integrated areas of each of these cis resonances are 2–3% of the corresponding trans resonance. Thus we have obtained essentially the same results as reported using ^1H NMR[1] indicating that the nuclear Overhauser enhancements are comparable for each of the two carbons in the cis and trans configuration.

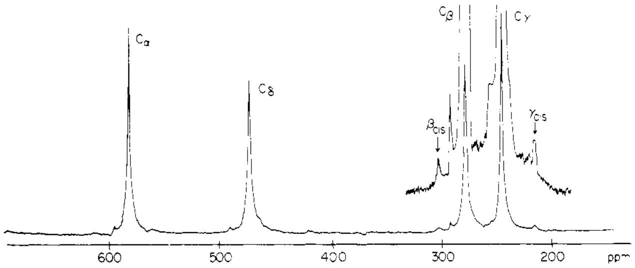

Figure 1. Partial carbon-13 Fourier-transform NMR spectrum at 67.9 MHz of poly(L-proline) (M = 9000) in D_2O at room temperature, 20 500 scans, 6.1 h total time. All spectra were obtained using a 4000 Hz total spectral width (8192 frequency domain points), a pulse repetition rate of 1.074 s, and an artificial broadening of 3 Hz due to exponential filtering to enhance sensitivity. Chemical shift scale is in ppm from Me_4Si. Arrows indicate cis resonance assignments.

With this confirmation, by means of carbon-13 spectroscopy, of the previous conclusions for poly(L-proline) similar

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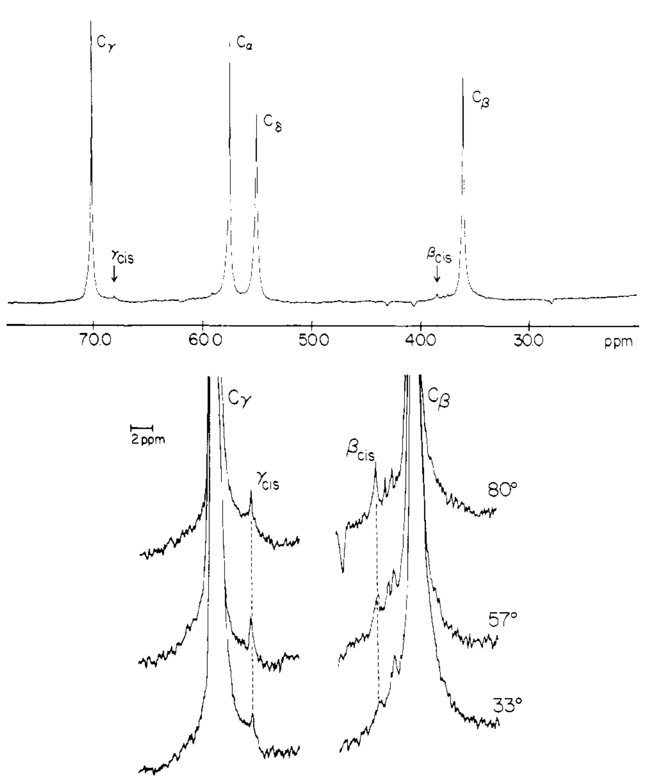

Figure 2. (a) Upper: Partial carbon-13 Fourier-transform NMR spectrum at 67.9 MHz of poly(γ-hydroxy-L-proline) in D_2O at 80 °C (M = 12 000), 30 000 scans, 9 h total time. Spectrum obtained under same conditions as in Figure 1. Arrows indicate cis resonance assignments. (b) Lower: Expansion of the region ± 5 ppm about the C^γ and C^β resonances of Figure 2a. The cis resonances and temperatures are indicated.

measurements were carried out with poly(γ-hydroxy-L-proline). Spectra were obtained successively at 33, 57, and 80 °C and then again at 33 °C. The initial spectra at 33 °C and the one obtained at this temperature after heating to 80 °C were identical indicating complete reversibility. A typical spectrum, at 80 °C, is given in Figure 2a. The four major resonances at 70.2, 57.6, 55.1, and 35.9 ppm downfield from Me_4Si were assigned respectively to the C^γ, C^α, C^δ, and C^β trans resonances based on the previous work of Torchia and Lyerla.[7] In this spectrum, there is a weak, but clearly discernable, resonance at 68.2 ppm. There are also several weak resonances between 1 and 2.3 ppm downfield from the C^β trans resonance. These weaker resonances are more clearly defined in Figure 2b which is an 8× linear expansion of the ±5 ppm region about the C^γ and C^β resonances. Qualitative changes in the relative intensity of these resonances with temperature is apparent.

Based on the previous ^{13}C assignment for the copolymer poly(γ-hydroxy-L-prolylglycyl)[7] and the comparison with the ^{13}C spectra of poly(γ-hydroxy-L-proline) in high concentration of aqueous solutions of LiBr and $CaCl_2$[9] the resonance at 68.2 ppm has been assigned to the C^γ cis configuration. By making a similar comparison with the homopolymer in the salt solutions[9] and with the copolymer[7] the resonance at 38.4 ppm has been assigned to C^β in the cis configuration.[19] As with poly(L-proline) the other minor resonances in the vicinity of C^β may be due to sequential effects of the cis isomer or another trans conformer.

The integrated intensities of the two assigned minor resonances for the C^γ and C^β, at 33 °C, are about 0.5–1.5% of the integrated intensities of the corresponding trans resonances. The percentage increases slightly with increasing temperature and is reversible, since the original percentage is regained when the temperature is lowered. If the Overhauser enhancement is the same in the cis and trans configuration for the two carbon atoms in question, as has been found for poly(L-proline), then the integrated intensities represent the proportion of cis imide residues present. This value is only slightly less than has been found for poly(L-proline). A similar series of spectra have been obtained for a polymer sample with twice the molecular weight of the one whose spectra are illustrated in Figures 2a and 2b. Except for a slight broadening of the resonance lines in the higher molecular weight sample, the two sets of spectra are essentially identical and yield the same relative percentages for the integrated intensities.

As has been indicated above, both poly(L-proline)[5] and poly(γ-hydroxy-L-proline)[4] have very similar dimensional properties in aqueous solution as deduced from their respective characteristic ratios which in turn must reflect very similar conformational energy maps for the two polymers. Conformational energy calculations, for either of the chains, when restricted to the all-trans configuration, which yield but a single minimum restricted to a narrow region of ψ (rotational angle about the C^α–C^1 bond), invariably yield very much higher characteristic ratios than are observed experimentally.[11,12] Therefore, other appropriate conformational space must be accessible to each of these homopolymer chains to account for the relatively low characteristic ratios that exist. Tanaka and Scheraga[3] have shown by Monte-Carlo calculation that for poly(L-proline) restricted to a narrow ψ region,[13] and presumably for poly(γ-hydroxy-L-proline) as well, the random introduction of a relatively small percentage of cis imide residues leads to a characteristic ratio consistent with experimental observation. Although these calculations are scattered similar conclusions can be reached by a more direct type of calculation.[14,15]

According to the calculations of Mattice, Nishikawa, and Ooi[16] for poly(L-proline) and Ooi, Clark, and Mattice[12] for poly(γ-hydroxy-L-proline) there is another region in conformational space, for the all-trans chains, which is available to both polymers and will also significantly reduce the characteristic ratio. These calculations yield two widely separated minima in ψ of similar energies and yield characteristic ratios in agreement with the experiments. However, in order for the other lower energy region to exist the pyrrolidine ring must possess some element of flexibility. There is sufficient evidence of flexibility in the proline ring.[17] A recent neutron diffraction study of γ-hydroxyproline[18] indicates that in the solid state its ring may also be quite flexible. Therefore, the distinct possibility also exists for the presence of the other ψ minimum for this chain.

In summary at the present time there is no unique explanation of the characteristic ratio and presumably other average properties of the two chains. The experimental data can be explained by the small amount of cis present, or a small occupancy of the second minimum, or a combination of both of these effects.[15] These alternative explanations all lie within theoretical and experimental error. One case that can be ruled out is the random interruption of the all-trans ordered helical structure. The concentration of cis residues required to lower the characteristic ratio is not compatible with the experimental results.[15]

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Semiempirical Distribution Functions for Linear and Circular Gaussian Chains

C. D. HOWELL and B. E. EICHINGER*

Department of Chemistry, BG-10, University of Washington, Seattle, Washington 98195.

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The known exact distribution functions of the three-dimensional radius of gyration for large linear chains[1-3] and for circular chains of any length[4] are complicated functions. In the first case, the distribution function $P(s)$ ds is given as an infinite sum of modified Bessel functions, and for the second case $P(s)$ ds for large chains is given by an infinite sum of polynomial and exponential functions of s^2. These functions are difficult to manage, since virtually all manipulations with them must be done numerically. Since distribution functions are applied to various problems,[5,6] it is expedient to find simple approximations to the exact functions which can be (i) easily manipulated and are (ii) reasonably accurate.

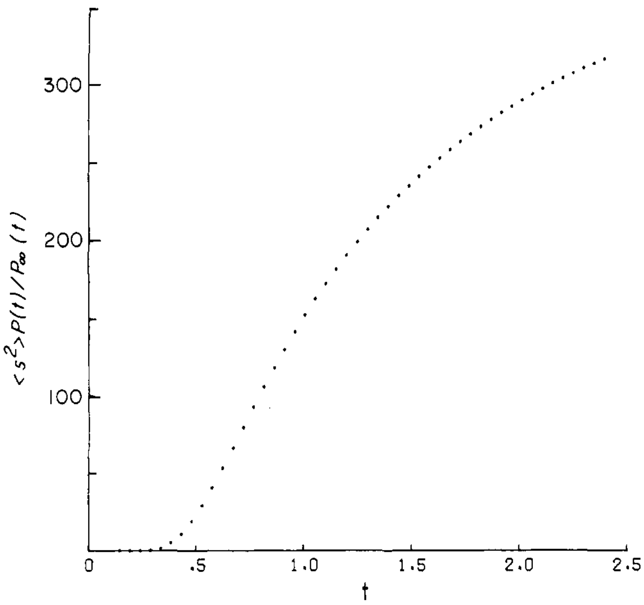

Figure 1. The unnormalized ratio $\langle s^2\rangle P(t)/P_\infty(t)$ for the circular chain.

Table I

Chain	Constant	a	β
Circular	405.9	0.74	5/4
Linear	11.68	0.23	17/10

The general features of distribution functions which have emerged recently[7] suggest that their binodal character is controlled by the asymptotic part of $P(s)$, here called $P_\infty(s)$. To test this conjecture, we have computed the exact distribution functions $\langle s^2\rangle P(t)$ dt, where $t = s^2/\langle s^2\rangle$ and $\langle s^2\rangle$ is the mean square (unperturbed) value of s, for both circular[3] and linear chains,[4] and have formed the ratio $P(t)/P_\infty(t)$. For the circular chain[4,7] the unnormalized

$$P_\infty(t) = t^2 \exp(-\pi^2 t/2) \qquad (1)$$

and for the linear chain[1,7] the corresponding function is

$$P_\infty(t) = t^{1/2} \exp(-\pi^2 t/4) \qquad (2)$$

The unnormalized ratio $P(t)/P_\infty(t)$ for the circular chain is shown in Figure 1. The corresponding function for the linear chain has similar behavior but is not shown. These functions bear strong resemblance to tanh $(1/t)$ as encountered in the Ising problem.[8] Thus, we were led to attempt to fit $P(t)$ by

$$\langle s^2\rangle P(t) = \text{const}[1 - \tanh (a/t^\beta)]P_\infty(t) \qquad (3)$$

where a and β are adjustable and $P_\infty(t)$ is given by eq 1 or eq 2 as appropriate. Values for the normalization constant, a, and β for both linear and circular chains selected to give the best fit near the maxima are given in Table I. Figures 2 and 3 graphically display the quality of the fit of the approximate functions to be exact. In Table II, the first few moments of t calculated from eq 3 are compared with exact values. The moments are generally accurate to within a few tenths of a percent. (The agreement between the approximate and exact moments could be made better by slight changes in a and β. However, specification of these parameters with an accuracy

Table II

Chain	Moment	Exact[a]	Approx	% dif
Circular	$\langle t\rangle = \langle s^2\rangle/\langle s^2\rangle$	1.0000	0.9982	0.2
	$\langle t^2\rangle = \langle s^4\rangle/\langle s^2\rangle^2$	1.1333 = 17/15	1.1296	0.3
	$\langle t^3\rangle = \langle s^6\rangle/\langle s^2\rangle^3$	1.4508 = 457/315	1.4430	0.5
Linear	$\langle t\rangle$	1.0000	1.0048	0.5
	$\langle t^2\rangle$	1.2667 = 19/15	1.2718	0.4
	$\langle t^3\rangle$	2.0032 = 631/315	2.0055	0.1
	$\langle t^4\rangle$	3.8698 = 1219/315	3.8603	0.3

[a] Exact moments for the circular chain were calculated from sums given by Šolc, ref 4, with expressions for cumulants given in ref 9. Values for the linear chain were taken from ref 5.

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