spectrum gradually with increasing spinning speed. Therefore, in the static spectrum of the 20% solution of $(BzlGlu)_n$ in $CDCl_3$, the line of the aromatic protons would be expected to be <300 Hz, that of benzyl protons 300–1000 Hz, of γ-CH_2 1–2.5 kHz, and that of β-CH_2 2.5–4.5 kHz wide. This line-width estimate is in good agreement with the overall shape of the static spectrum. From this it is evident that the static spectrum is a superposition of separate bands of different width, justifying the neglect of intergroup interactions, as compared to intragroup interactions, in the first approximation. In discussing the relaxation mechanism manifested by the shape of separate chemically shifted bands, only the intragroup dipolar interactions of the respective proton group need be considered.

Whenever line narrowing by MAR is observed, at least some interactions must conform to the condition $\omega_r\tau_c > 1$. From the narrowing of the phenyl band already at ω_r approaching 10^3 sec^{-1}, it can be assumed that the slow mechanism causing the broadening of lines in the 20% solution of $(BzlGlu)_n$ in $CDCl_3$ must have a $\tau_c > 10^{-3}$ sec. We assume that this slow mechanism corresponds to isotropic reorientations of helix axes in the solution of liquid crystal type. By dilution, the constraints on the motion of the helix axes are loosened, and for this reason dilution leads to a similar result as MAR. When such a quality of spectrum has been obtained by dilution that it cannot be further narrowed by MAR, then it can be concluded that the reorientation of helix axes in the dilute solution is isotropic with a correlation frequency exceeding the highest macroscopic spinning frequency used in the experiment.

From the shape of the static spectrum of a 20% solution of $(BzlGlu)_n$ in $CDCl_3$ and from the process of its narrowing with increasing spinning speed, the static width of the band assigned with great probability to β-CH_2 protons is estimated to be 2.5–4.5 Hz. If, in addition to the very slow reorientation of helix axes, the rapid rotation of the β-CH_2 group about the C_α–C_β bond were the only relaxation mechanism of this group, then the resulting line width would have to be of the order of one-half of the width of the rigid lattice doublet,[14] *i.e.*, much larger than observed. This implies that the β-CH_2 group must participate in some other rapid motion, in addition to the rotation about the C_α–C_β bond. Such an additional rapid motion could be represented by rotation about the helix axis, with a correlation time $\tau_c < 1/\omega_r = 2 \times 10^{-4}$ sec.

For the narrowing of bands of α-CH and NH protons, with a static line width of 15–20 kHz, neither the attainable macroscopic spinning frequencies nor the correlation frequencies of isotropic helix reorientations in dilute solution are sufficient. The necessary frequencies can evidently only be reached when segmental motion of the polymer backbone sets in, as in the random-coil conformation.

The residual line widths corresponding to side-chain proton groups are limited by rapid motional mechanisms, especially by reorientations of the respective group about the sequence of axes represented by the system of chemical bonds in the side chain. It may be expected that the correlation time of these motions will decrease with increasing distance of the group from the main chain, in agreement with the observed decreasing limiting line width of the corresponding lines. Simultaneously, however, with the increasing complexity of the anisotropic motion of increasingly more distant groups, the geometrical factors A, B, etc., in the line-width expressions (eq 1 and 3) are expected, from Woessner's analysis, to gradually change. Therefore the correlation frequencies of motions of the separate proton groups of the side chain cannot be determined quantitatively without a detailed analysis of the motional mechanism and calculation of the geometrical factors Such a calculation for $(BzlGlu)_n$ and some simpler model systems will be the subject of a subsequent communication.

(14) E. R. Andrew and R. A. Newing, *Proc. Phys. Soc. London*, 72, 959 (1958).

Carbon-13 and Proton Nuclear Magnetic Resonance Observations of the Conformation of Poly(L-proline) in Aqueous Salt Solutions

Douglas E. Dorman, Dennis A. Torchia, and Frank A. Bovey*

Bell Laboratories, Murray Hill, New Jersey 07974. *Received October 11, 1972*

ABSTRACT: Proton nmr at 220 MHz and ^{13}C nmr at 25 and 15.08 MHz have been employed to confirm previous conclusions that the disordering of poly(L-proline) chains in concentrated aqueous salt solutions arises primarily from the formation of random sequences of cis and trans peptide bonds. The effects of KI and $CaCl_2$ are essentially similar. The β- and γ-carbon resonances of the proline ring appear to be the most reliable monitors of this isomerization process, giving distinct and well resolved peaks for the cis and trans conformations.

The disordering of poly(L-proline) chains in concentrated aqueous salt solutions has been interpreted as arising from either (a) an increase in the accessible range of the C_α—C=O angle ψ,[1-5] or (b) the formation of random sequences of cis and trans peptide bonds.[6-9] Kurtz and Harrington[10] have ascribed the loss of regular structure to a combination of increased excursions of ψ and small deviations of the peptide bond from planarity. Johnston and

(1) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, E. Katchalski, *J. Amer. Chem. Soc.*, 82, 5263 (1960).
(2) T. Schleich and P. H. von Hippel, *Biopolymers*, 7, 861 (1969).
(3) P. H. Von Hippel and T. Schleich, *Accounts Chem. Res.*, 2, 257 (1969).
(4) M. L. Tiffany and S. Krimm, *Biopolymers*, 6, 1767 (1968).

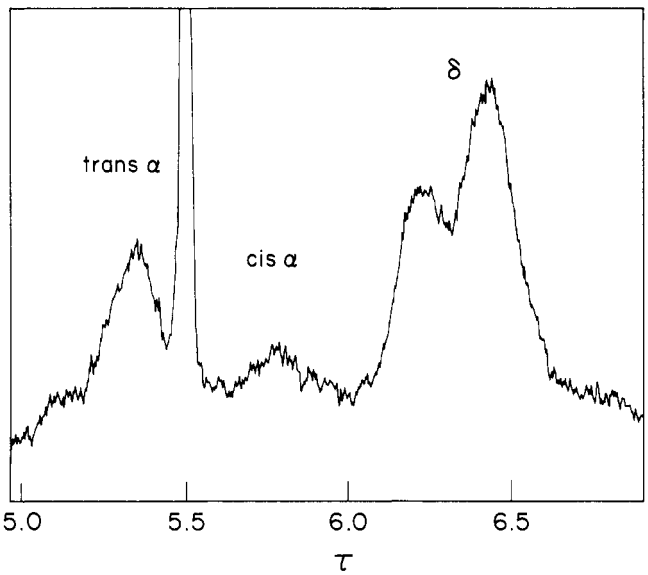

Figure 1. 220-MHz proton spectrum of poly(L-proline) of *ca.* 10,000 molecular weight in 4 *M* $CaCl_2$ solution in D_2O at 58°; the narrow resonance at τ 5.5 is that of residual HDO.

Krimm[5] and Swenson[9] reported that the infrared spectrum of form I (*i.e.*, all cis) shows an increase in the carbonyl frequency over form II (all trans) as well as small changes in the ring C—H bending frequency.[5,9] Essentially identical spectral results were, however, interpreted in opposite fashion by these investigators.

In an earlier study we interpreted the high-resolution proton magnetic resonance (pmr) spectrum of poly(L-proline) in terms of the cis–trans peptide bond hypothesis (b).[8] The α-proton resonances of salt solutions of poly(L-proline) were very broad. This was believed to result from the random distribution of the cis–trans sequences, as is observed for polysarcosine.[11] Interpreted in this way, this peak broadening provides significant information. But it also presents problems of quantitative interpretation. The area of the cis α-proton peak appears deceptively small if line shape and baseline are not properly taken into account. Johnston and Krimm[5] have accordingly challenged our interpretation, suggesting that at most only residues at or near the end of the chain become cis in the presence of the salt. If this is indeed only an end effect, then as pointed out by these authors the relative intensity of the cis resonance would be inversely proportional to the molecular weight. A test of this point appears in Figure 1, in which is shown the 220-MHz proton spectrum of a poly(L-proline) of *ca.* 10,000 molecular weight in 4 *M* $CaCl_2$ at 58°; this spectrum is essentially unchanged from that obtained earlier,[8] although the molecular weight of the polymer is about four times greater.

Interpretation would be more straightforward if the resonances of the cis and trans isomers were not overlapping. This is true for at least part of the ^{13}C magnetic resonance (cmr) spectrum. In Figure 2 are shown the cmr spectra of the ring carbons of aqueous solutions of the same preparation of poly(L-proline) (mol wt *ca.* 2500) used in the earlier work.[10] The assignment of carbon resonances is aided by a more extensive survey of acyclic proline derivatives,[12] from which it was concluded that the chemical shift of the γ carbon was a reliable indicator of cis–trans isomerism at the peptide bond. In Figure 2a is presented the spectrum of poly(L-proline) I, taken immediately after dissolution in cold water (9°), under which conditions earlier kinetic studies indicate that the isomerization should be strongly retarded.[8] As expected, the spectrum shows only one major isomer. The chemical shift of the γ carbon, which in this case *must* correspond to the cis isomer, is in agreement with conclusions of the model studies.[12] Spectrum 2c is that of a solution in which the isomerization has been allowed to proceed completely to form II. The γ-carbon shift is consistent with that of the trans isomers of the models. In Figure 2b is shown the cmr spectrum of poly(L-proline) I after it has been allowed to partially (*ca.* two-thirds) isomerize to form II. Both forms are clearly evident, and their β and γ resonances are well resolved. In Figure 2d is shown a spectrum of poly(L-proline) initially in form II, dissolved in 5 *M* aqueous KI and observed at about 25°. It is obvious that spectra b and d are virtually identical except for the slight broadening and somewhat lower signal-to-noise ratio of the latter.

^{13}C spectra obtained at 15.08 MHz in aqueous and $CaCl_2$ solutions are shown in Figure 3 for a sample having

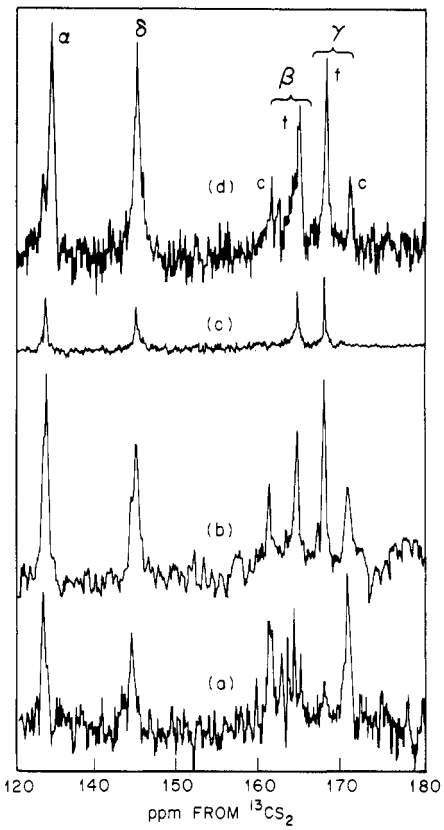

Figure 2. 25-MHz ^{13}C spectra of form I poly(L-proline) of *ca.* 2500 molecular weight; (a) immediately after dissolution in H_2O at 9°; the multiplet centered near 163 ppm is that of acetone-d_6; (b) same as part a but allowed to isomerize partially (*ca.* ⅔) to form II; (c) after complete isomerization to form II; (d) same polymer dissolved as form II in 5 *M* aqueous KI, observed at 25°.

(5) N. Johnston and S. Krimm, *Biopolymers*, **10**, 2597 (1971).
(6) W. F. Harrington and M. Sela, *Biochim. Biophys. Acta*, **27**, 24 (1958).
(7) W. L. Mattice and L. Mandelkern, *J. Amer. Chem. Soc.*, **93**, 1769 (1971).
(8) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).
(9) C. A. Swenson, *Biopolymers*, **10**, 2591 (1971).
(10) J. Kurtz and W. F. Harrington, *J. Mol. Biol.*, **17**, 440 (1966).
(11) F. A. Bovey, J. J. Ryan, and F. P. Hood, *Macromolecules*, **1**, 305 (1968).
(12) D. E. Dorman and F. A. Bovey, in preparation.

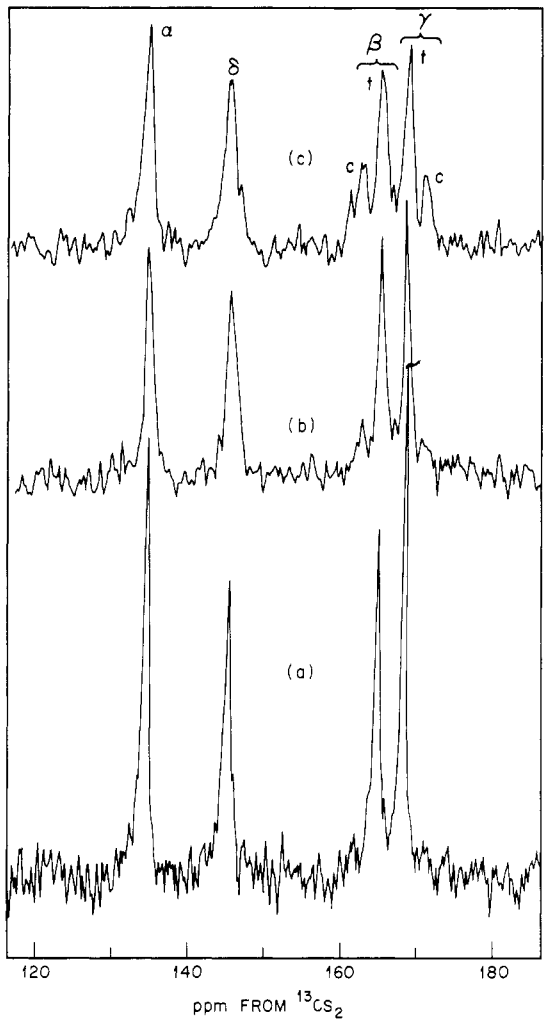

Figure 3. 15.08-MHz ^{13}C spectra of poly(L-proline) of *ca.* 11,000 molecular weight; (a) dissolved as form II in H_2O; (b) in 1.8 *M* $CaCl_2$ in H_2O; (c) in 4.0 *M* $CaCl_2$ in H_2O. Spectra observed at 60°.

a molecular weight of *ca.* 11,000. It is clear that the relative intensities of the minor β- and γ-carbon resonances increase with increasing $CaCl_2$ concentration and that in 4 *M* $CaCl_2$ the poly(L-proline) spectrum is very similar to that in 5 *M* KI (Figure 2d). The principal difference between the spectra is the increased width of the lines in $CaCl_2$ solution, which is probably attributable to the higher molecular weight of the polymer and the higher viscosity of $CaCl_2$ solutions as solvent even at the higher temperature.

As implied in the above discussion, Johnston and Krimm[5] believe that our earlier results[8] can be satisfactorily explained in terms of a model involving only trans peptide bonds, except possibly at the chain ends. We believe that the data presented here demonstrate that both the proton and carbon resonances, observed at the expected cis positions, can be explained only if these bonds are distributed along the entire chain. The hypothesis that chains contain cis and trans bonds also explains (a) the observation of two sets of resonances and (b) the small characteristic ratio.[7] A somewhat greater freedom of rotation about the C_α—C=O- bond[5] cannot account for either of these observations. If one postulates the presence of both cis′ (ψ = 120°) and trans′ (ψ = 300°) in an all trans chain, *i.e.*, very large variations in ψ, there is then a barrier sufficiently high, according to calculation, to account for separate resonances for the conformations[13,14] and a sufficient collapse of the structure to account for the observed characteristic ratio.[15] But it is difficult to see how the very high-energy cis′ state could be significantly populated.

The most serious objection to this interpretation, however, is that it requires that the carbon chemical shifts known to correspond to a cis-trans′ (*i.e.*, form I) conformation in aqueous solution coincide exactly in aqueous salt solutions with the quite different trans-cis′ conformation. Such coincidences are extremely rare in ^{13}C spectroscopy. We therefore believe that we have strengthened our previous conclusion that the conformational disordering of poly(L-proline) in aqueous salt solutions results from the presence of random sequences of cis and trans peptide bonds.

Experimental Section

The cmr spectra were measured in aqueous solutions at 25 MHz on a Varian XL-100 spectrometer which has been adapted for pulse Fourier transform spectroscopy,[16] and at 15.08 MHz on a spectrometer built at the National Bureau of Standards. Carbon chemical shifts were measured relative to internal 1,4-dioxane, and were subsequently referred to external carbon disulfide on the basis of the chemical shift of 1,4-dioxane relative to that standard (126.2 ppm).

The pmr spectrum was taken under conditions which have been previously described.[8]

The poly(L-proline) of mol wt 11,000 was purchased from the Research Division of Miles Laboratories, Inc. The sources of the other two samples are given below.

Acknowledgment. We acknowledge several very helpful conversations with Dr. A. E. Tonelli in the course of this work. We thank Drs. C. M. Deber and E. R. Blout for the sample of poly(L-proline) of mol wt 2500 and Drs. W. L. Mattice and L. Mandelkern for the material of mol wt 10,000.

(13) P. R. Schimmel and P. J. Flory, *Proc. Nat. Acad. Sci. U. S.*, **58**, 42 (1967).
(14) P. R. Schimmel and P. J. Flory, *J. Mol. Biol.* **34**, 105 (1968).
(15) A. E. Tonelli, private communication.
(16) H. Sternlicht and D. M. Zuckerman, *Rev. Sci. Instrum.*, **43**, 525 (1972).