of the subfragment II sections) they can be eliminated on the basis of our findings on the geometry of the LMM and rod dimers.

Acknowledgments

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Evidence for Cis Peptide Bonds in Copolypeptides of Glycine and Proline†

D. A. Torchia

ABSTRACT: Poly(Pro-Gly) and poly(Gly-Gly-Pro-Gly) have been examined using 220-MHz nuclear magnetic resonance (nmr) spectroscopy. The data indicate that the two polypeptides have unordered structures in trifluoroethanol, dimethyl sulfoxide, and aqueous solution. However, the spectra of both sequential copolypeptides show two resonances, having unequal areas, for individual Gly NH protons and Pro α protons. Evidence is presented which shows that these resonances result from the presence of both cis and trans Gly–Pro peptide bonds "randomly" distributed in a given polypeptide chain. The observed temperature dependence of the relative population of the two isomers in water yields approximate values of the enthalpy and entropy difference between the cis and trans isomers. Solvent also influences the relative population of the isomers, with the fraction of cis bonds in poly(Pro-Gly) decreasing from 0.4 to 0.2 (at 22°) on going from dimethyl sulfoxide to aqueous solution. Solvent effects on line widths and NH chemical shifts suggest that the flexibility of the polypeptide backbone and the solvent accessibility of Gly NH protons are solvent dependent. In the light of these results, the conformational characteristics of naturally occurring polypeptides containing proline are discussed.

Recent nuclear magnetic resonance (nmr) studies (Madison and Schellman, 1970; Deber *et al.*, 1970; Torchia *et al.*, 1972a,b) have shown that linear and cyclic oligopeptides containing Pro[1] residues maintain multiple conformations in solution distinguished by different isomers (cis and trans) of X-Pro peptide bonds. Except for poly(Pro) itself, cis peptide bonds have not been reported in solution for polypeptides containing Pro residues. In addition to having possible biological applications, the observation of cis X-Pro peptide bonds in polypeptides and measurement of effects of solvent and temperature on the population of cis isomers would be useful in aiding interpretation of optical and hydrodynamic data. Also, such results can be used to check calculated polypeptide conformational energies. High-frequency nmr is ideally suited to detect the presence of cis and trans peptide isomers. The barrier to cis $\rightleftarrows$ trans interconversion is sufficiently high so that rotation about the peptide bond is slow and separate resonances, corresponding to the two isomers, can be detected directly (Torchia and Bovey, 1971).

For these reasons we have chosen to study poly(Pro-Gly) and poly(Gly-Gly-Pro-Gly) using 220-MHz nmr. A previous study (Mattice and Mandelkern, 1970, 1971a,b) of these polymers, using optical and hydrodynamic techniques, has shown that they are unordered in trifluoroethanol and aqueous solution. In the unordered state, rapid rotation about the single bonds in the polypeptide backbone occurs which results in averaging out of direct dipolar interactions. Thus, high-resolution spectra, which greatly simplify analysis, are

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[1] The following abbreviations will be used in this paper: Gly for glycyl; Pro for L-prolyl; Ser for L-seryl; Sar for sarcosyl; X for any amino acid residue except prolyl; *t* for temperature; DP for degree of polymerization; Me_4Si for tetramethylsilane; Me_2SO-d_6 for hexadeuteriodimethyl sulfoxide.

TABLE I: Molecular Weights[a] of Poly(Pro-Gly) and Poly(Gly-Gly-Pro-Gly).

Copolypeptide	$10^{-3} M_n$	$10^{-3} M_w$
Poly(Pro-Gly)	8.0 ± 0.4	13.2 ± 0.3
Poly(Gly-Gly-Pro-Gly)	4.9 ± 0.5	6.7 ± 0.2

[a] From Mattice and Mandelkern (1971b).

obtained for both polypeptides at room temperature even though each has DP ~ 100. Two other features which simplify interpretation of the spectra are (a) the lack of a Pro NH resonance which means only Gly NH resonances are observed in the downfield region of the spectrum, and (b) Pro and Gly α resonances are well separated which allows observation of the Pro $C_\alpha H$ resonance without interference from Gly $C_\alpha H_2$ resonances.

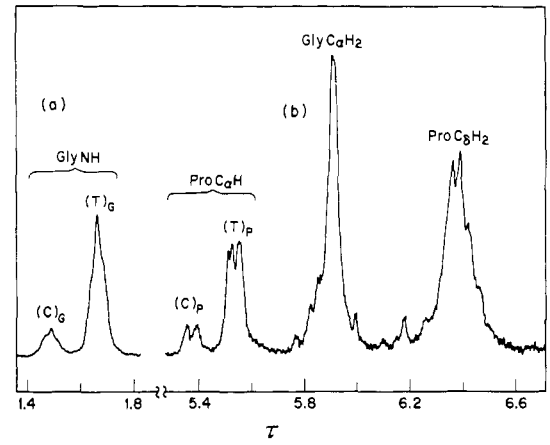


FIGURE 1: 220-MHz spectrum of poly(Pro-Gly) (a) at 22°, in H_2O–CH_3COOH, 99.5:0.5 in the NH region of the spectrum and (b) at 22° in D_2O–CD_3COOD, 99.5:0.5 by volume in the τ region 5.3–6.7 of the spectrum. Concentration: 30 mg/ml in a and 20 mg/ml in b. Chemical shifts (τ scale) given in ppm from *tert*-butyl-d_1 alcohol at τ 8.77.

Materials and Methods

Sequential Copolypeptides. Poly(Pro-Gly) and poly(Gly-Gly-Pro-Gly) were kindly supplied by Professor D. F. DeTar. Their synthesis, by the *p*-nitrophenyl ester method (DeTar and Vajda, 1967; DeTar *et al.*, 1967a,b), was accomplished by polymerization of the *p*-nitrophenyl ester of the indicated sequence and they were purified by exhaustive dialysis. Both weight average (M_w) and number average (M_n) molecular weights of these polymers have been determined (Mattice and Mandelkern, 1971a,b) and are summarized in Table I.

Nuclear magnetic resonance spectra were obtained on Varian HR-220 and HA-100 spectrometers. In some instances, the signal-to-noise ratio was improved by accumulation of data in Fabri-Tek (at 220 MHz) and Varian (at 100 MHz) time-averaging computers having 1024 channels. Temperature of the samples was controlled by a Varian temperature control unit. In trifluoroethanol and dimethyl-d_6 sulfoxide (Me_2O-d_6), tetramethylsilane (Me_4Si) at τ 10.0 was used as an internal reference. In aqueous solution, the *tert*-butyl resonance of *tert*-butyl-d_1 alcohol at τ 8.77 (relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate) was used as internal reference. In preparing samples in D_2O, the polymer was first dissolved in 99.8% D_2O and the sample was then frozen and lyophilized in the nmr tube. After evaporation of the D_2O, the tube was transferred to a dry box and the sample was dissolved in Diaprep "100.0%" D_2O. Using this procedure, the area of the HDO resonance was reduced to *ca.* 20% of the area of the Pro $C_\alpha H$ resonance and HDO spinning side bands are absent from the spectra.

Results and Discussion

Poly(Pro-Gly) Spectra in Aqueous Solution. Figure 1a shows the downfield (τ 1.4–1.8) region of the poly(Pro-Gly) spectrum obtained in H_2O–CH_3COOH, 99.5:0.5 by volume. Two Gly NH resonances, designated $(T)_G$ and $(C)_G$, are seen having relative areas of *ca.* 4:1 at 22°. Hence, the $(C)_G$ resonance accounts for 20% of the Gly NH's and cannot be ascribed to end effects since DP ~ 100. The observation of two Gly NH resonances indicates that the Gly NH protons in the polymer backbone experience two distinct magnetic environments. This result is of interest since it shows that the polymer does not maintain an unordered conformation with all peptide bonds trans, for then, all Gly residues would experience the same (average) magnetic environment.

Examination of the poly(Pro-Gly) spectrum, Figure 1b, in the upfield (τ 5.3–6.7) region shows that two distinct Pro $C_\alpha H$ resonances, designated $(T)_P$ and $(C)_P$, are also present. As found for the two NH resonances, the relative areas of the $(T)_P$ and $(C)_P$ resonances are in the ratio of *ca.* 4:1. The asymmetry of the Gly $C_\alpha H_2$ resonances also indicates at least two overlapping AB quartets are present since a single AB pattern is symmetric. The complexity of the Pro $C_\delta H_2$ resonances (at *ca* τ 6.4) and Pro $C_\beta H_2$ and Pro $C_\gamma H_2$ resonances (at τ 7.7–8.1 but not shown) does not permit resolution of the (C) and (T) resonances corresponding to these protons.

The possibility that the (T) and (C) resonances result from an equilibrium between aggregated and nonaggregated structures was ruled out since a tenfold change in concentration (3 mg/ml to 30 mg/ml, at 21°) produced no change in $\mathcal{R}_{C/T}$, the area of (C) resonance/area of (T) resonance. However, $\mathcal{R}_{C/T}$ was found to change reversibly with temperature; a semilogarithmic plot of $\mathcal{R}_{C/T}$ *vs.* $(Rt)^{-1}$ appears in Figure 2.

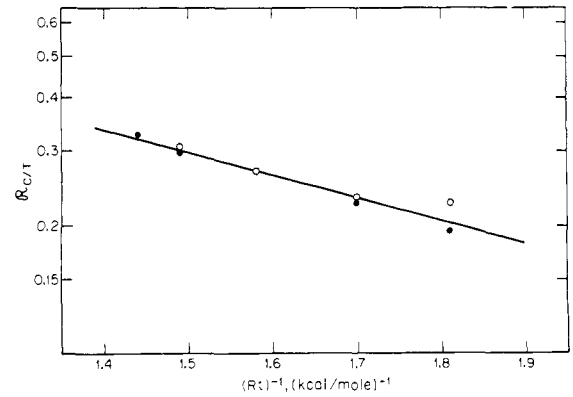


FIGURE 2: Semilogarithmic plot of $\mathcal{R}_{C/T}$, ratio of area of (C) resonance to that of (T) resonance, *vs.* $(Rt)^{-1}$. Open circles represent values of $\mathcal{R}_{C/T}$ computed from Gly NH resonances. Solid circles were computed from Pro $C_\alpha H$ resonances. Uncertainty in measurements *ca.* ±10%.

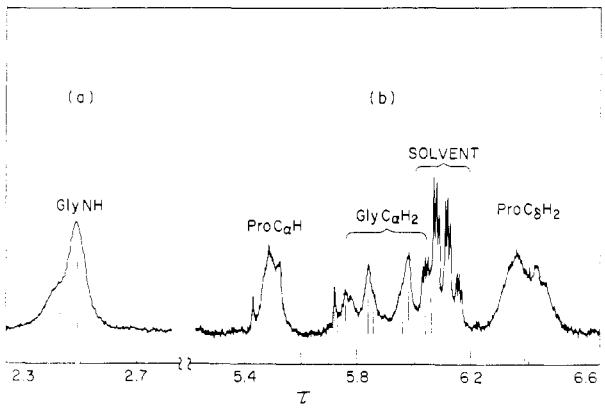
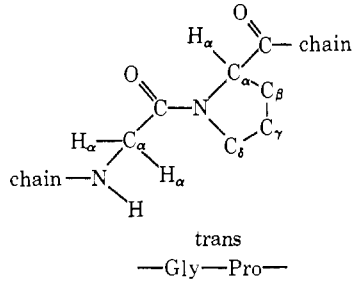


FIGURE 3: 220-MHz spectrum of poly(Pro-Gly) (a) at 3°, in trifluoroethanol in the NH region and (b) at 44°, in trifluoroethanol-d_3 in the τ 5.3–6.6 region. Concentration 20 mg/ml in a and 20 mg/ml in b. Chemical shifts (τ scale) in ppm from tetramethylsilane at τ10.0. In b the shaded resonances at τ 5.42 and 5.72 are the upfield spinning sidebands of the residual solvent OH resonance (at τ 5.15), while the multiplet labeled "solvent" is the CF_3CHDOD resonance.

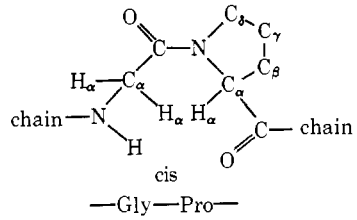
The increase of $\Re_{C/T}$ as temperature increases indicates that the (C) resonances correspond to an energetically less favorable conformation (than (T)), and on increasing the temperature the relative population of the (C) conformation increases.

The free energy barrier to interconversion of (T) and (C) conformations is at least 15 kcal/mole since separate resonances are observed at 80°. This suggests that (T) $\rightleftarrows$ (C) interconversions require rotation about the peptide bond, which is known to have an activation enthalpy of 20–25 kcal/mole (Downie and Randall, 1959; Steinberg *et al.*, 1960). Studies (Madison and Schellman, 1970; Deber *et al.*, 1970; Torchia *et al.*, 1972a,b) on linear and cyclic oligopeptides containing proline have shown that these compounds maintain multiple conformations (in equilibrium) distinguished by different isomers (cis or trans) of the X–Pro peptide bond. Diagrams of cis and trans Gly–Pro peptide bonds, of interest in the present study, are shown below. The oligomer studies (Deber



(I)

trans
—Gly—Pro—



(II)

cis
—Gly—Pro—

et al., 1970; Torchia *et al.*, 1972b) indicate that (a) the cis X–Pro $C_\alpha H$ resonance falls downfield of trans in polar solvents and (b) trans X–Pro peptide bonds predominate in water and trifluoroethanol. For these reasons, we assign the predominant $(T)_G$ and $(T)_P$ resonances to Gly NH and Pro $C_\alpha H$ protons associated with trans Gly–Pro peptide bonds and the $(C)_G$ and $(C)_P$ resonances to corresponding protons in cis Gly–Pro peptide bonds. It is reasonable to ask what effects the different isomers at a given Gly–Pro peptide bond have on the chemical shifts of the Pro and Gly protons in residues which are on either side of the -Gly-Pro- unit considered. While such effects are expected to be small, the apparently poor resolution observed for Gly NH resonances in Figure 1a and the asymmetry of the $(C)_G$ resonance (the poor resolution and asymmetry were observed on repeated measurements over the 0–60° temperature range on single and computer-averaged scans) indicate that they are not completely negligible. A full discussion of this matter is provided following the analysis of the poly(Gly-Gly-Pro-Gly) spectra.

It is possible to estimate the differences in enthalpy and entropy of the cis and trans Gly–Pro bonds in the chain, since the linear dependence of $\ln \Re_{T/C}$ on $(Rt)^{-1}$ seen in Figure 2 suggests that a simple two-state analysis of the data is approximately correct. Thus we write

$$\ln \Re_{C/T} = \Delta S/R - \Delta H/Rt \qquad (1)$$

where $\Delta S = S_C - S_T$, the difference in entropy of cis and trans Gly–Pro peptide bonds, $\Delta H = H_C - H_T$, the difference in enthalpy of cis and trans Gly–Pro peptide bonds. From the straight line in Figure 2 one finds $\Delta H \approx 1.2$ kcal/mole and $\Delta S \approx 1.3$ eu/mole.

In writing eq 1 it is assumed that S_T and H_T are the same for every trans Gly-Pro isomer in the polypeptide, with analogous remarks applying to S_C and H_C. This approximation would not be valid if the polypeptide contained predominantly cis bonds. In such cases, models indicate a high probability that the chain would fold back on itself resulting in steric interactions between sequentially remote residues. S and H would then depend upon the number of cis bonds in the chain as well as interactions at an individual peptide bond. Under the conditions studied, poly(Pro-Gly) contains less than 15% cis bonds and it is reasonable to interpret the values of ΔS and ΔH obtained from Figure 2 as resulting from differences between short-range interactions at cis and trans Gly–Pro peptide bonds.

Poly(Pro-Gly) Spectra in Trifluoroethanol. Two NH resonances are seen in the spectrum of poly(Pro-Gly) in trifluoroethanol (Figure 3a). The center positions of these resonances, at τ 2.42 and 2.49, are indicated by the vertical lines under the resonances. Evidence for two types of $C_\alpha H$ resonances is seen in Figure 3b. The Gly $C_\alpha H_2$ resonances are represented by the two sets of overlapping AB spectra whose positions are given by the lines under the Gly $C_\alpha H_2$ resonances. The most downfield resonances of the Gly $C_\alpha H_2$ AB patterns (at τ 5.76–5.78) are seen resolved into two distinct resonances (the separation of these resonances was observed repeatedly on subsequent scans). The asymmetric Pro $C_\alpha H$ resonance is also indicative of overlapping (symmetric) individual Pro $C_\alpha H$ resonances, and the unequal areas of the Pro $C_\delta H_2$ resonances cannot be explained if only one resonance for each C_δ proton is present. These considerations show that two resonances are present corresponding to each Gly and Pro proton and once again these resonances are assigned to protons in cis and trans Gly–Pro peptide bonds with the trans isomer predominating. The greater separation of cis and trans poly(Pro-Gly) resonances found in aqueous solution is in accord with results obtained for poly(Pro) II and di-

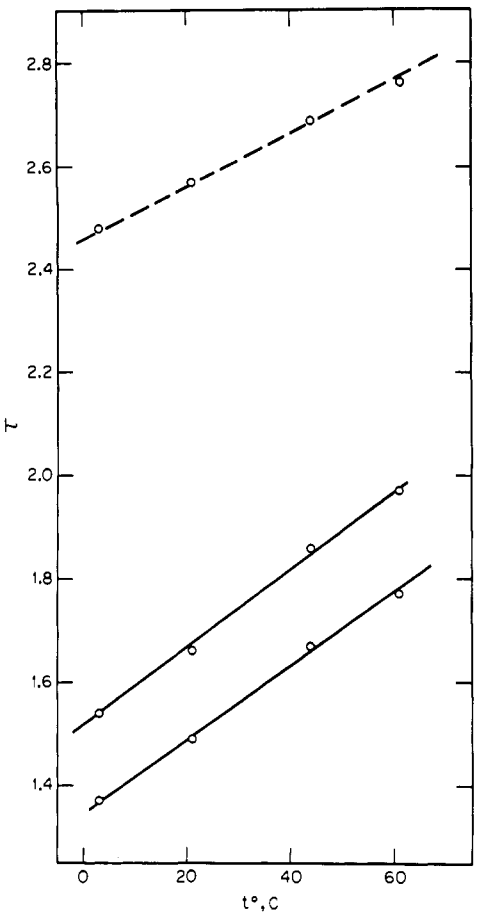


FIGURE 4: Temperature dependence of poly(Pro-Gly) NH resonances in trifluoroethanol (- - -) and in H_2O–CH_3COOH, 99.5:0.5 by volume (—). The virtually identical chemical shift dependence exhibited by the two Gly NH resonances in trifluoroethanol is represented by a single dashed line. Chemical shifts (τ scale) measured in ppm from Me_4Si in trifluoroethanol and from *tert*-butyl-d_1 alcohol (at τ 8.77) in aqueous solution.

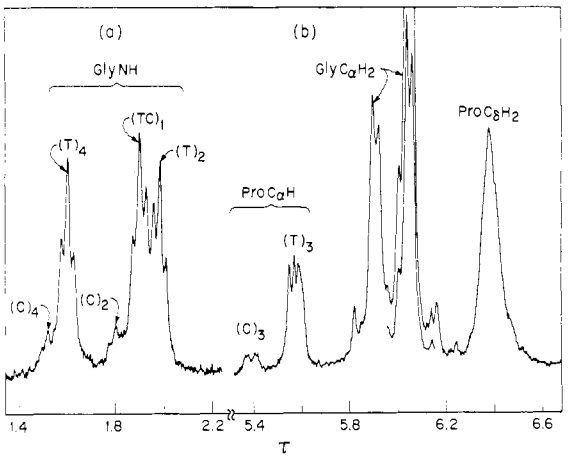


FIGURE 5: 220-MHz spectrum of poly(Gly-Gly-Pro-Gly) (a) at 44°, in H_2O–CH_3COOH, 99.5:0.5 by volume in the NH region and (b) at 22°, in D_2O–CD_3COOD, 99.5:0.5 by volume in the τ 5.4–6.6 region of the spectrum. Concentration: 30 mg/ml in a and 25 mg/ml in b. Gly $C_\alpha H_2$ resonances at *ca.* τ 6 are shown at lower amplification under the main spectrum. Chemical shifts (τ scale) in ppm from *tert*-butyl-d_1 alcohol at τ 8.77.

methylformamide. In aqueous solution the cis and trans Pro $C_\alpha H$ resonances of poly(Pro) II are separated by 0.3 ppm while the methyl resonances of dimethylformamide are 0.15 ppm apart. In trifluoroethanol these separations are reduced to 0.2 and 0.1 ppm in the case of poly(Pro) form II and dimethylformamide, respectively.

Overlap of the resonances prevents accurate measurement of the relative population of the two types of isomers; however, a computer simulation of the Gly NH region of the spectrum (at 3°) suggests relative areas of *ca.* 3:1 at 3°, rather than *ca.* 5:1 as found in aqueous solution at 3°. In addition to the well-known case of poly(Pro), solvent effects on population of cis and trans isomers have also been found in linear dipeptides (Madison and Schellman, 1970) and cyclic hexapeptides (Torchia *et al.*, 1972a,b) containing Pro residues.

Solvent effects on line widths are also found on going from aqueous solution to trifluoroethanol with the result that, at all temperatures in the range 0–60°, poly(Pro-Gly) spectra are always better resolved in aqueous solution than in trifluoroethanol. Poly(Pro) II resonances (at 22°) are also better resolved in aqueous solution. Both poly(Pro-Gly) and poly(Pro) II show larger values of intrinsic visosity in trifluoroethanol than in water, and it has been proposed (Mattice and Mandelkern, 1970, 1971a) that in trifluoroethanol the low energy region of the conformational energy (φ,ψ) map (Edsall *et al.*, 1966a–c; Brant *et al.*, 1967; Schimmel and Flory, 1968) is more restricted than in aqueous solution, leading to a "stiffer" polypeptide chain. The nmr results are in accord with this conclusion.

Behavior of Poly(Pro-Gly) NH Resonances. Since the polypeptide is in an unordered conformation in water and trifluoroethanol, the Gly NH's are exposed and hydrogen bonded to solvent hydroxyl oxygens. Upfield shifts of hydrogen-bonded protons result (Eyman and Drago, 1966) on decreasing the strength of the hydrogen bond. Increasing the temperature decreases the average strength of the hydrogen bond between the Gly NH's and solvent since the populations of the higher energy hydrogen bonding conformations increases. The upfield shifts of the poly(Pro-Gly) NH protons, shown in Figure 4, are thus in accord with these considerations. All NH's exhibit linear upfield shifts having slopes (5.4–7.4 $\times$ 10^{-3} ppm/°C) which are in agreement with slopes found for cyclic hexapeptide (Torchia *et al.*, 1972a,b) NH's exposed to polar solvents.

Poly(Gly-Gly-Pro-Gly) in Aqueous Solution. The spectrum of the α and δ protons of poly(Gly-Gly-Pro-Gly) is shown in Figure 5b. Two Pro $C_\alpha H$ resonances, designated $(T)_3$ and $(C)_3$ are observed in the spectrum which have virtually the same fine structure, chemical shifts, and relative areas as found for the Pro $C_\alpha H$ resonances of poly(Pro-Gly) in aqueous solution (Figure 1b). Measurement of areas of $(T)_3$ and $(C)_3$ resonances (uncertainty $\pm 10\%$) show that $\mathcal{R}_{C/T}$ increases from 0.17 at 22° to 0.23 at 75°. On the basis of our discussion of the poly(Pro-Gly) spectrum, the $(T)_3$ and $(C)_3$ resonances in Figure 5b are assigned to Pro α protons in trans and cis Gly–Pro peptide bonds, respectively.

Assignment of the five Gly NH resonances which are seen in Figure 5a is more difficult; however, a self-consistent set of assignments can be made. For purposes of discussion the residues in the polymer repeat sequence are numbered as shown

$$\text{-Gly}_1\text{-Gly}_2\text{-Pro}_3\text{-Gly}_4 \qquad \text{(III)}$$

We note that the five NH resonances in Figure 5a are separated into two well-defined groups. The downfield

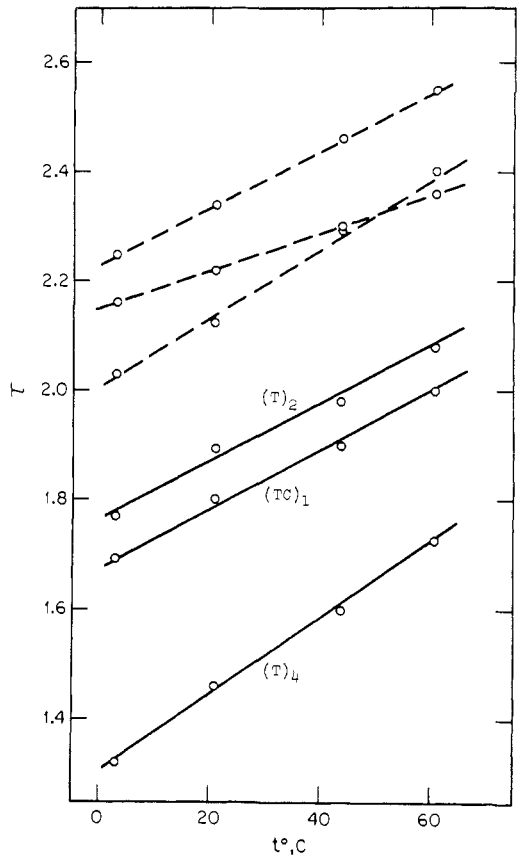


FIGURE 6: Temperature dependence of poly(Gly-Gly-Pro-Gly) NH resonances in trifluoroethanol (- - -) and in H_2O–CH_3COOH, 99.5:0.5 by volume (—). The linear upfield shifts of the Gly $(C)_2$ and $(C)_4$ resonances (not shown) parallel those of $(T)_2$ and $(T)_4$, respectively. Chemical shifts (τ scale) measured in ppm from Me_4Si in trifluoroethanol and from *tert*-butyl-d_1 alcohol (at τ 8.77) in aqueous solution.

pair, designated $(T)_4$ and $(C)_4$, are assigned to the NH of Gly_4 since this NH has the unique property of forming a peptide bond with a Pro carbonyl, unlike the NH's of Gly_1 and Gly_2, both of which form peptide bonds with Gly carbonyl groups. We note that the chemical shifts of $(T)_4$ and $(C)_4$ are very similar to that of the $(T)_G$ and $(C)_G$ NH resonances of poly(Pro-Gly) (see Figure 1a), in which the Gly NH also forms a peptide bond with the Pro carbonyl. The chemical shift difference between $(T)_4$ and $(C)_4$ is about one-third of the 0.2 ppm difference between $(T)_G$ and $(C)_G$. This is understandable since in poly(Gly-Gly-Pro-Gly) it is the Gly_2–Pro_3 peptide bond which is cis or trans and one expects the Gly_2 NH resonances (rather than the more remote Gly_4 NH's) corresponding to cis and trans Gly_2-Pro_3 isomers to show the 0.2-ppm chemical shift difference. On examination of the upfield group of NH resonances in Figure 5a we see that $(T)_2$ and $(C)_2$ are separated by 0.2 ppm. Therefore, these resonances are assigned to the Gly_2 NH's in trans and cis Gly_2-Pro_3 isomers.

By elimination, the remaining resonance, $(TC)_1$, is assigned to the NH of Gly_1. Since the Gly NH resonances are found to satisy the relationships, area of $(TC)_1 \approx$ area of $(T)_2$ + area of $(C)_2$, area of $(TC)_1 \approx$ area of $(T)_4$ + area of $(C)_4$, it follows that the chemical shift of the Gly_1 NH is not affected by the nature of Gly_2–Pro_3 peptide bond. This result is reasonable since the Gly_1 NH is separated from the Gly_2–Pro_3 peptide bond by six intervening bonds. It should be noted that these considerations indicate that the chemical shift of an NH proton is measurably dependent on the isomer at the peptide bond of which it is part, as well as the isomers at the preceding and succeeding peptide bonds. Hence, as has been concluded from a study of poly(Sar) (Bovey *et al.*, 1968) a triad type analysis is required to account for NH or NCH_3 resonances observed when cis and trans peptide bonds are present.

We now return to the discussion of the Gly NH resonances observed in Figure 1a. Two Pro-Gly sequences, IV and V, distinguished by different Gly_2-Pro_3 isomers, will be considered.

$$\text{-Pro}_1'\text{-Gly}_2'\text{-Pro}_3'\text{-Gly}_4'\text{-} \quad \text{(cis)} \qquad \text{(IV)}$$

$$\text{-Pro}_1'\text{-Gly}_2'\text{-Pro}_3'\text{-Gly}_4'\text{-} \quad \text{(trans)} \qquad \text{(V)}$$

Note that the numerical subscripts are primed to distinguish the residues from those in the III sequence of Gly-Gly-Pro-Gly. Assuming that Gly_4'–Pro peptide bond is trans in IV and V (a similar argument applies if it is cis) the Gly_4' NH protons in IV and V will exhibit slightly different chemical shifts, similar to the chemical shift difference observed for the $(T)_4$ and $(C)_4$ resonances of poly(Gly-Gly-Pro-Gly), which in this latter case also results from the perturbation of the Gly_4 NH chemical shifts (in III) due to cis and trans isomers of Gly_2–Pro_3 peptide bonds in (III). This leads to the apparent poor resolution and asymmetry of the $(T)_G$ and $(C)_G$ resonances in Figure 1a. In contrast to this result, well-resolved Pro $C_\alpha H$ resonances, $(T)_P$ and $(C)_P$ are observed in Figure 1b, since the Pro_1' residue is sufficiently remote from the Gly_2'–Pro_3' peptide bond (note that it occupies the position in IV and V analogous to the Gly_1 position in III) so that the Pro_1' $C_\alpha H$ chemical shifts are not significantly influenced by the nature of the Gly_2'–Pro_3' peptide bond.

It should be emphasized that while the above considerations logically account for all the data, the Gly assignments must be regarded as tentative until a specifically labeled sample (*e.g.*, Gly_2 or Gly_3 deuterated) is examined.

Poly(Gly-Gly-Pro-Gly) in Trifluoroethanol. Separate resonances, corresponding to cis and trans isomers of Gly–Pro peptide bonds, were not resolved in the spectra of poly(Gly-Gly-Pro-Gly) in trifluoroethanol or in trifluoroethanol-d_3. Since there is no reason to expect only one Gly–Pro peptide bond isomer to be present, this result is most likely due to the difficulty of observing the small separation of the rather poorly resolved resonances in these solvents.

Behavior of NH Resonances. Since poly(Gly-Gly-Pro-Gly) has an unordered structure in both aqueous and trifluoroethanol solution, linear upfield shifts of all NH resonances were expected on increasing temperature. As seen in Figure 6, the anticipated results are obtained in aqueous solution where all resonances show linear upfield shifts (slopes 5.4–7.4 $\times$ 10^{-3} ppm/°C). In trifluoroethanol two of the three Gly NH's shift as expected; however, the shift coefficient of one resonance has an anomalously small value of 3.2 $\times$ 10^{-3} ppm/°C. These results indicate that one Gly NH is somewhat solvent shielded in trifluoroethanol but not in water. Thus, as has been suggested from hydrodynamic studies (Mattice and Mandelkern, 1971a), the unordered conformations are not identical in the two solvents.

Spectra in Me_2SO-d_6. In view of the differences in behavior of the polypeptides in trifluoroethanol and aqueous solution and the small separation of resonances due to cis and trans isomers in the organic solvent, spectra were also obtained in Me_2SO-d_6. Portions of the 220-MHz spectrum of poly(Pro-Gly) in Me_2SO-d_6 at 22° are shown in Figure 7a. The two Gly NH resonances, $(C)_G$ and $(T)_G$, and two Pro $C_\alpha H$ resonances, $(C)_P$ and $(T)_P$, show that cis and trans Gly-Pro isomers are present. Of greater interest is the fact that the fraction of cis bonds, 0.4 at 22°, is about twice the value measured in aqueous solution. Thus, as found in the case of cyclic(Ser-Pro-Gly-Ser-Pro-Gly) (Torchia *et al.*, 1972b), Me_2SO-d_6 stabilizes cis X–Pro peptide bonds more effectively than water. On addition of water to the Me_2SO-d_6 solution, the fraction of cis bonds decreases, attaining a value of about 0.2 (at 22°) when the water mole fraction is *ca.* 0.5. The re-equilibration of isomer populations on mixing solvents occurs in less time than required to obtain a spectrum (*ca.* 3 min). Both NH resonances, $(C)_G$ and $(T)_G$, exhibit linear upfield shifts, with slopes of 6.7×10^{-3} and 5.8×10^{-3} ppm/°C, respectively, on increasing the temperature.

Evidence for cis Gly–Pro peptide bonds is also seen in the portions of the poly(Gly-Gly-Pro-Gly) spectrum (in Me_2SO-d_6, 22°) shown in Figure 7b. The assignments were made by analogy with the similar spectra obtained in aqueous solution and shown in Figures 5a and 5b. Again the fraction of cis bonds is about twice that found in water. On increasing the temperature, the Gly NH resonances show linear upfield shifts, with slopes in the range 5 to 5.5 $\times 10^{-3}$ ppm/°C. Hence, evidence for a partially shielded NH of poly(Gly-Gly-Pro-Gly) is found only in trifluoroethanol. Line widths in Me_2SO-d_6 are broader than in aqueous solution, suggesting the presence of a more flexible structure in water.[2]

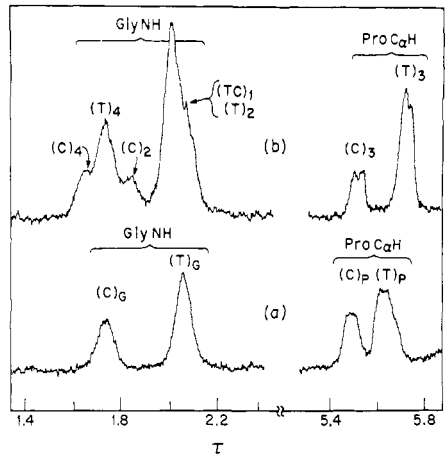
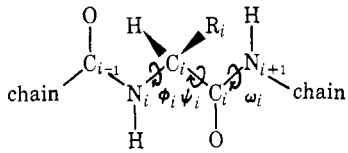


FIGURE 7: Portions of 220-MHz spectra of (a) poly(Pro-Gly) and (b) poly(Gly-Gly-Pro-Gly) showing Gly NH and Pro $C_\alpha H$ resonances in Me_2SO-d_6 at 22°. Concentration: 30 mg/ml in a and b. Chemical shifts (τ scale) in ppm from Me_4Si at τ 10.0.

Conclusions

To specify the secondary structure of a polypeptide, knowledge of all residue rotation angles, (φ,ψ,ω) (Edsall *et al.*, 1966a–c), is required. These angles are illustrated below for the i'th residue in a polypeptide chain. Both poly(Pro-Gly)



and poly(Gly-Gly-Pro-Gly) are in unordered conformations under the conditions studied, therefore one is limited to a statistical description of the structure of these polypeptides. In the classical approximation (Brant *et al.*, 1967; Schimmel and Flory, 1968), the relative probability of finding the i'th residue with rotation angles in the range $d\varphi_i d\psi_i d\omega_i$ is given by $P(\varphi_i,\psi_i,\omega_i) = \exp[-E(\varphi_i,\psi_i,\omega_i)/Rt]d\varphi_i d\psi_i d\omega_i$, where E is the rotational potential energy.

Segmental motion in polypeptides results from changes in (φ,ψ) angles due to rapid rotation about N—C_α and C_α—C=O (single) bonds. Figures 1 and 5 show that poly(Pro-Gly) and poly(Gly-Gly-Pro-Gly) yield high-resolution spectra, with line widths comparable to those found for oligopeptides. Thus, there is a high degree of segmental motion in these unordered polypeptide chains in water, resulting in short correlation times characteristic of small peptides. Correlation times and hence rotational potential functions are solvent dependent with broader resonances found in Me_2SO-d_6 and trifluoroethanol, indicating longer correlation times in the organic solvents. Additional evidence of solvent effects on the rotational potential function comes from the anomalously small temperature coefficient of one poly(Gly-Gly-Pro-Gly) NH resonance in trifluoroethanol (but not in aqueous or Me_2SO-d_6) solution.

The rapid rotation about N—C_α and C_α—C=O bonds contrasts with the slow rotation (on the nmr time scale) about the peptide bond. This slow rotation results in separate spectra for the two isomers allowing direct measurement of their relative populations. The analysis of the temperature dependence of $\mathcal{R}_{C/T}$ (Figure 2) indicates the rotational potential for a cis Gly–Pro peptide bond, $E(\phi_i,\psi_i,\omega_i)$, $\omega_i = 180°$, is broader but shallower than the potential for the trans isomer, $E(\phi_i,\psi_i,\omega_i)$, $\omega_i = 0°$, in aqueous solution.

The small free energy differences between cis and trans Gly-Pro isomers in these polymers suggest that biologically active polypeptides containing Pro residues may also have cis X–Pro peptide bonds. Since the trans $\rightleftarrows$ cis conversion involves a 180° change in ω, introduction of even a single cis peptide bond in a naturally occurring polypeptide is expected to result in a significant change in conformation and hence biological activity. On the basis of solvent-induced changes in population of cis and trans isomers of cyclo(Ser-Pro-Gly-Ser-Pro-Gly) it has been suggested (Torchia *et al.*, 1972b) that the biological activity of naturally occurring cyclic oligopeptides may be controlled, in some instances, by environmentally induced changes in conformation resulting from 180° rotations about X–Pro peptide bonds. The solvent dependence of $\mathcal{R}_{C/T}$ reported here indicates that these remarks also apply to naturally occurring linear polypeptides containing Pro residues.

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[2] A comparison of line widths in Figure 7a with those in 7b shows that poly(Pro-Gly) Pro-$C_\alpha H$ resonances (in Me_2SO-d_6) are broader than poly(Gly-Gly-Pro-Gly) Pro $C_\alpha H$ resonances. This is not the case in aqueous solution as seen from Figures 1 and 5, indicating that in sequences IV and V, the nature of the $Gly_{2'}$–$Pro_{3'}$ peptide bond influences the chemical shift of the $Pro_{1'}$ $C_\alpha H$ proton to a greater degree in Me_2SO-d_6 than in water.

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Cyanogen Bromide Cleavage of Guinea Pig Skin Collagen. Isolation and Characterization of Peptides from the α1 and α2 Chains†

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ABSTRACT: After CNBr cleavage of the chromatographically purified α1 and α2 chains of guinea pig skin collagen (GPSC), eight peptides were isolated from each chain. These peptides were resolved by a combination of ion-exchange and gel filtration chromatography, and characterized by determination of their amino acid composition and molecular weight. The 16 peptides obtained in this manner adequately account for the amino acid composition and molecular weight of the respective intact chains. In contrast to the collagens of other species, the α1 and α2 chains of GPSC display a different number and distribution of methionyl residues. In the α1 chain, a methionine is missing in the sequence homologous to α1-CB7 plus α1-CB6 of other species; while in the α2 chain, two additional methionines exist in the sequence homologous to α2-CB4 of other species. Consequently, the chromatographic patterns of CNBr peptides, particularly those obtained from the α2 chain, differ considerably from those of other collagens. GPSC is, nevertheless, clearly similar to other collagens in overall amino acid composition and in molecular weight. This study provides a base line for subsequent investigations which utilize the CNBr peptides of GPSC, particularly those designed to determine the distribution of intermolecular cross-links in the insoluble protein.

Cleavage of collagen at methionyl residues with cyanogen bromide (CNBr) has proven to be a useful technique in a variety of studies aimed at characterizing this important macromolecule. Such studies include the comparative biochemistry of collagen chains from different tissues and species (see Piez *et al.* (1968) for a review), and the localization and characterization of an intramolecular covalent cross-link in the protein (Bornstein and Piez, 1966; Bornstein *et al.*, 1966; Kang *et al.*, 1969c). Recently this approach was directed to an analysis of insoluble collagen in an attempt to isolate and identify peptides containing intermolecular cross-links (Miller, 1971; Volpin and Veis, 1971a).

These experiments were initiated in an attempt to establish a good model system for the investigation of insoluble collagen. In order to interpret the complex pattern of peptides obtained

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