phonate oxygens, O(6) and O(8), act as acceptors in the hydrogen bonding. O(6) of the phosphonate is simultaneously involved in an acceptor hydrogen bond involving the solvent. The second hydrogen atom of the amino group and site N(7) are engaged in a base–phosphonate pairing scheme to a symmetry related phosphonate group. In this pairing, therefore, the Hoogsteen sites in the base, N(6) and N(7), are involved. The ribose hydroxyl O(5′)H donates a hydrogen bond to the solvent and accepts a hydrogen bond from the O(2′)H group of another ribose. Thus the hydrogen bonding involving the following five nonhydrogen atoms forms a closed loop, –O(2′)–H → O(5′)–H → O(9)–H → O(6) ← H–N(1)– (Figure 3).

The only potential hydrogen-bonding site on the base that is not involved in hydrogen bonding is N(3). As seen here and in other structures, position N(7) in the adenine and guanine bases shows a strong tendency for hydrogen bonding. Therefore, in double-helical nucleic acids, although this site is not engaged in the Watson–Crick base pairing scheme, it probably is involved in hydrogen bonding to solvent molecules. To a lesser extent this site shows metal coordination properties, and may be bonded to monovalent metal ions such as Na^+ or divalent metal ions such as Zn^{2+} (for a review, see ref 17).

The hydrogen bonding in this structure varies from approximately linear to markedly nonlinear, the angles being in the range 137.0–175.4° (Table VI).

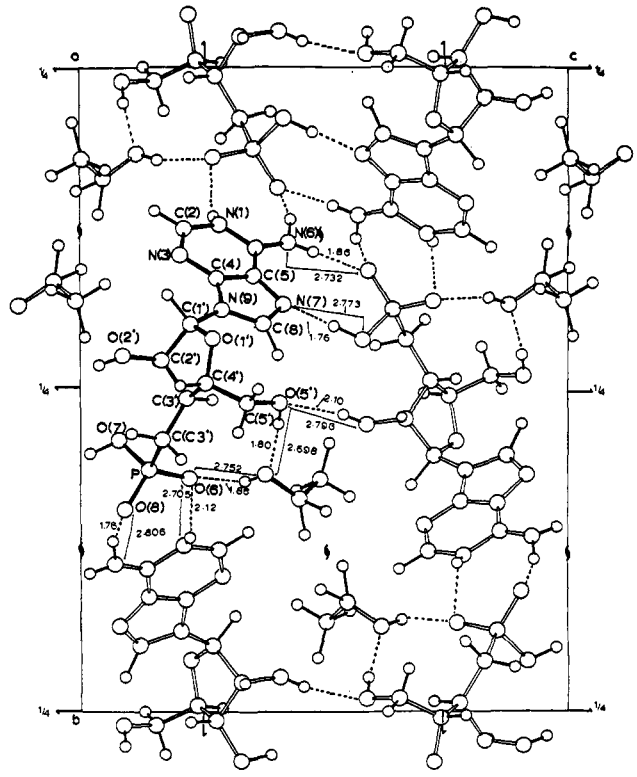

Figure 4. Hydrogen bondings cheme projected down the *a* axis and the hydrogen bond distances. The molecule in solid bonds depicts the reference molecule.

Acknowledgment. It is a pleasure to thank Dr. J. G. Moffatt and Dr. G. H. Jones, Institute of Molecular Biology, Syntex Research, Palo Alto, Calif., for supplying the crystals and Dr. S. T. Rao, Department of Biochemistry, University of Wisconsin, for helpful discussions during the course of this work. Financial support of this work by the National Institutes of Health of the United States Public Health Service Grant 17378 and the Wisconsin Alumni Research Foundation is gratefully acknowledged.

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Spectroscopic Characterization of Poly(Ala-Gly-Gly)

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Contribution from the Division of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106. *Received August 10, 1971*

Abstract: Poly(Ala-Gly-Gly) was synthesized in our laboratory as part of a study concerning the role of tripeptide sequences, omitting proline, in the structure of collagen. Three forms of the polymer, two ordered and one disordered, have been isolated depending on solvent and temperature. These have been characterized using ultraviolet absorption and circular dichroism spectroscopy in conjunction with infrared-absorption and linear dichroism spectroscopy. Form I, with a cross β conformation, was isolated from dichloroacetic acid or acetic acid. Form II, with a 3_1-helical conformation, was found in dilute aqueous solution or could be film cast from this solvent, whereas form III, a disordered form, is found in hot aqueous or 6 *M* calcium chloride solutions, as well as in films cast from trifluoroacetic acid or hot water. This polytripeptide is therefore apparently the first which is known to have a stable 3_1 helix in aqueous solution and which does not contain proline. Furthermore the thermal melting is the first observation of a polyglycine II helix–random coil transition.

Spectroscopic characterization of synthetic polyamino acids has yielded much available information which has subsequently been applied to conformational aspects of both fibrous and globular proteins. More recently sequential polypeptides have been synthesized and studied as models for fibrous proteins such as silk and collagen. Whereas much of the early work concentrated on the β sheet and α helix (usually right handed) and transitions of the latter (helix–coil), much of the recent work has concentrated on the left-hand 3_1 helix of poly-L-proline and its derivatives and the distorted supercoiled 3_1 helix found in proline-containing

polytripeptides of the form $(Gly\text{-}Pro\text{-}X)_n$; these polytripeptides generally resemble the collagen structure in the solid state.[1]

Although collagen also contains, in its primary sequence, glycine-led triads without proline, relatively little work has been performed with similar synthetic materials, although extensive studies of poly(Ala-Glu(OEt)-Gly) have been reported from our own laboratory.[2,3] This material has been found only in one form (cross β) so far.

Poly(Ala-Gly-Gly) is unusual in that it has at least three different possible conformations, including one which is apparently the first example of a left-handed 3_1 helix known for a polytripeptide which does not contain proline.

Brack and Spach[4] have presented a partial characterization of poly(Ala-Gly-Gly) using infrared spectroscopy and preliminary X-ray diffraction data. Their work suggested that two forms are obtainable depending on the solvent history; form I obtained from organic acids such as DCA was assigned an extended β conformation while the second form, obtained from aqueous media, was said to consist of polyglycine II helices. Subsequently[5,6] more detailed studies substantiated the presence of these two forms in the solid state. In an earlier communication we presented evidence for the presence of the 3_1 helix of form II in dilute aqueous solutions of the polymer.[7] This was based on the similarity between the circular dichroism spectrum of the solution and that obtained by film casting from the aqueous solution. The spectrum was also similar to that for other known 3_1-helical polypeptides and, in addition, there was evidence of a heat-induced conformational change, consistent with a helix to disordered transition. The circular dichroism spectrum of the latter was similar to that proposed by Tiffany and Krimm for disordered polypeptides[8] rather than the "charged coil" of polyglutamic acid so often used as a random coil model. This paper, then, presents a more detailed characterization of the three forms of poly(Ala-Gly-Gly) using spectroscopic techniques and discusses the implications of the observed structures.

Experimental Section

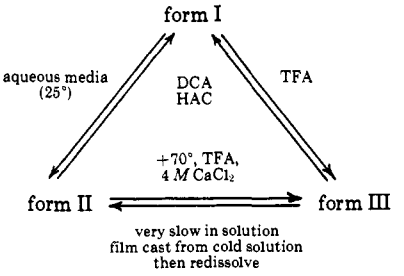
The polymer was synthesized in our laboratory by Dr. J. M. Anderson from the *p*-nitrophenyl ester of the monomeric tripeptide Ala-Gly-Gly. The polymer used for the melting experiment was fractionated on a 2 cm × 40 cm column packed with Sephadex G50 obtained from Pharmacia Fine Chemicals. This column was calibrated with protein markers from the same company and a fraction with molecular weight ~5000–6000 was used. Due to the limited supply of polymer, all other experiments used the unfractionated material.

Infrared-absorption spectra were taken on a Perkin-Elmer 521 grating spectrophotometer. Linear dichroism measurements were made using a wire grid polarizer mounted in the common beam, in front of, and at 45° to the entrance slits. Oriented films were obtained by the method outlined previously[2] and the spectra recorded with the stroking direction aligned parallel and perpendicular to the plane of polarization.

Ultraviolet circular dichroism and optical rotatory dispersion spectra were recorded on a Cary 60 spectropolarimeter with a Model 6001 circular dichroism attachment. The melting studies on form II utilized a jacketed 1-cm cell (Luminon Inc. Type 032) coupled to a Haake Model FT circulating water bath. Spectra were recorded at 15-min intervals after reaching the desired temperature. Spectra were taken until there were no further changes. The circular dichroism spectra reported for films were obtained by casting the films directly onto quartz disks. The spectrum from the polymer in 6 *M* $CaCl_2$ was obtained using a 0.1-mm path length demountable cell from Hellma Cells Inc. Ultraviolet-absorption spectra were taken with a Cary 11 spectrophotometer and all curve resolving utilized a DuPont 310 curve resolver.

Results and Discussion

The methods used to obtain the various forms of the polymer are summarized below.



DCA = dichloroactic acid
TFA = trifluoroacetic acid
HAC = acetic acid
form I = β
form II = polyglycine II
form III = disordered

Molecular Weight Determination. Table I summarizes the results of several independent methods for characterization of molecular weight. Although there is considerable discrepancy between the various methods some of this may be attributable to the low solubility of the polymer in water—a fact that was not taken into consideration when the end group titration was performed. However, it seems safe to assume a $M_n \geq 5000$ which is adequate (in polypeptide work) to class the materials as polymer rather than oligomer. The weight-average molecular weight value is probably somewhat less than two times the number-average molecular weight expected for this method of polymerization due to the removal of lower molecular weight material during purification; this results in a molecular weight distribution with a sharp cut off toward the low molecular weight end. Although restrictions on sample precluded a more detailed analysis of molecular weight,

Table I. Molecular Weight Determinations Using Various Methods of Determination

Method	Type of molecular wt	Value	Degree of polymerization	Solvent
Amino end group titration	$\overline{M_w}$	14,100[a]	69	H_2O
p-Nitrophenyl ester spectrophotometric determination	$\overline{M_w}$	8,400	41	DCA
Gel filtration on calibrated Sephadex	$\overline{M_w}$	9,350	46	H_2O
$[m]^4D$		>4,000	>20	H_2O

[a] See ref 7.

(1) N. S. Andreeva, N. G. Esipova, M. I. Millinova, V. N. Rogulenkova, and V. A. Shibnev, *Mol. Biol.*, **1**, 657 (1967).
(2) W. B. Rippon, J. M. Anderson, and A. G. Walton, *J. Mol. Biol.*, **56**, 507 (1971).
(3) J. C. Andries and A. G. Walton, *ibid.*, **56**, 515 (1971).
(4) A. Brack and G. Spach, "Peptides," North Holland Publishing Co., Amsterdam, 1968.
(5) J. C. Andries, J. M. Anderson, and A. G. Walton, *Biopolymers*, **10**, 1049 (1971).
(6) H. D. Keith and B. Lotz, *J. Mol. Biol.*, **61**, 195 (1971).
(7) W. B. Rippon and A. G. Walton, *Biopolymers*, **8**, 347 (1969).
(8) M. L. Tiffany and S. Krimm, *ibid.*, **8**, 347 (1969).

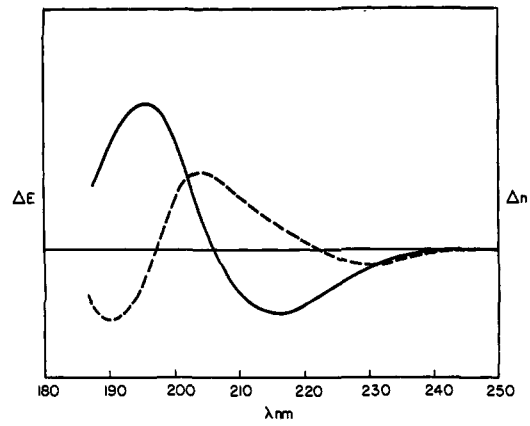


Figure 1. Circular dichroism (—) and optical rotatory dispersion (----) spectra for films of poly(Ala-Gly-Gly) cast from dichloroacetic acid or precipitated from acetic acid.

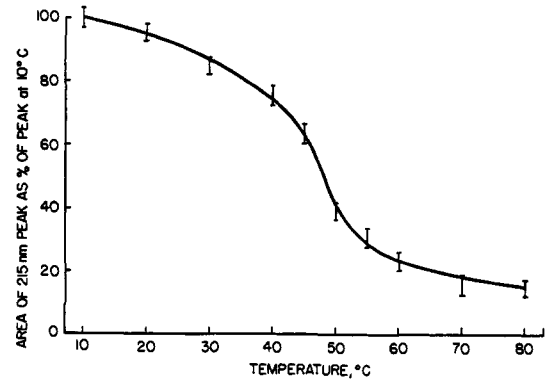


Figure 2. Temperature dependence of the 3_1 helix–disordered state transition of poly(Ala-Gly-Gly) in H_2O.

the fact that our results appear to be in agreement with other studies on this polymer[4,6] where a molecular weight of 14,100 was reported suggests that there is probably very little distortion due to inadequate polymerization.

Ultraviolet-Absorption and Circular Dichroism Spectroscopy. Although decisive identification of form I as a cross β conformation with antiparallel chains depends on the infrared dichroism studies reported later in this paper, the circular dichroism spectrum and the optical rotatory dispersion spectrum reported in Figure 1 are consistent with this interpretation. The spectra were obtained from films and are in accord with other similar studies involving β structures,[9] yet on their own do not give any information about chain orientation.

The conversion from form II to form III can, as noted above, be monitored using circular dichroism spectroscopy.

It is accompanied by a considerable reduction in the intensity of both the positive band at ~213 mμ and the negative band at ~192 mμ as well as a slight red shift. The transition can be induced by heating the dilute aqueous solution and evidence has been presented for considering it to involve a helix to disordered conformational change.[7] As such it may be described as a "melting" of the polyglycine II helix.

Figure 2 shows the melting curve obtained by monitoring the area under the positive circular dichroic band as a function of temperature. This area was obtained after resolving the spectrum into a positive gaussian followed by a negative gaussian. Such resolution becomes more critical for the higher temperature curves since the rather small positive band is considerably distorted by the adjacent trough. The area at temperature T is plotted as a fraction of the area at temperature $T_0 = 10°$ and a melting curve with a relatively broad transition and a skewing at the low temperature end is obtained.

The skewing of the curve toward the low temperature end along with the broad melting profile makes evaluation of a specific melting temperature rather difficult. The conventional approach of selecting the temperature midway between that which gives 25% and that which results in 75% of the total change in monitored variable results in a T_m of 44°. However, this value is distorted due to the unsymmetrical nature of the melting curve; an alternative would be to select the temperature giving a value midway between tangents drawn to the initial and final slopes of the curve since these are approximately parallel. This method gives a T_m of ~48°. The skewing of the curve at the low-temperature end may be due to less than perfect order in the 10–30° range. This would then mean that further cooling could result in additional order with the area of the circular dichroism approaching a limiting value rather slowly. A similar gradual decrease in order would occur on raising the temperature causing a relatively slow drop in circular dichroism band intensity for the initial stages. This interpretation is consistent with the known effects of ordered sequence length on circular dichroism spectra as shown by oligomer studies.[10] This explanation, which amounts to saying that the transformation is noncooperative, would also explain the broad melting curve since it assumes the presence of intermediate states. However it should be mentioned that the curves obtained at various temperatures were isodichroich within the level of experimental error and this would favor a two-state model. Finally, circular dichroism curves similar to those obtained from the heated aqueous solution were also obtained for poly(Ala-Gly-Gly) in 6 *M* $CaCl_2$ and films cast from hot water, trifluoroacetic acid, and formic acid. Curves similar to the PGII form could be obtained by film casting from acetic acid as distinct from the β form obtained by precipitation from this solvent on cooling. Adler, *et al.*,[11] have reported circular dichroism curves similar to that obtained for poly(Ala-Gly-Gly) form II—the trough at ~230 mμ and the reduced ellipticity at ~218 mμ could be due to the presence of less than perfect 3_1 helices in what they describe as the random coil form.[12] Finally, the mirror image of the curve we assign to a left-handed 3_1 helix has been reported for poly-*N*-methylalanine,[13] a polymer, which, conformational calculations suggest, exists as a right-handed 3_1 helix.[14]

(9) L. Stevens, R. Townend, S. N. Timasheff, G. D. Fasman, and J. Potter, *Biochemistry*, **1**, 3717 (1968).

(10) *E.g.*, H. Akabayashi, H. Isemura, and S. Sakakibeva, *Biopolymers*, **6**, 323 (1968).

(11) A. J. Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.*, **90**, 4737 (1968).

(12) M. L. Tiffany and S. Krimm, *Biopolymers*, **6**, 1379 (1968).

(13) M. Goodman and M. Fried, *J. Amer. Chem. Soc.*, **89**, 1264 (1967).

(14) J. E. Mark and M. Goodman, *ibid.*, **89**, 1267 (1967).

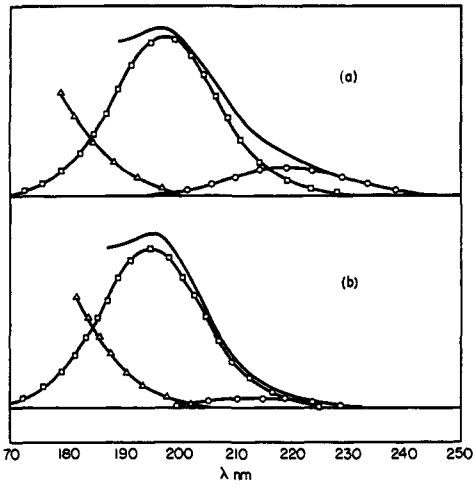


Figure 3. Ultraviolet absorption spectrum (—) for poly(Ala-Gly-Gly) in disordered form (a) and the 3_1 helix. The component gaussians are shown for the n–π^* transition (-○-), the π_1–π^* transition (-□-), and the π_2–π^* transition (-△-).

The similarity between the changes in circular dichroism spectra for this transition of poly(Ala-Gly-Gly) and that observed for the collagen–gelatin transition is noteworthy. This latter transition has been followed by Gratzer[15] using far-ultraviolet absorption spectroscopy and he noted a hyperchromic effect in both the n→π^* and the π_1–π^* transition upon denaturation with a larger effect on the n–π^* transition. McMillin, *et al.*,[16] have reported monitoring the vacuum ultraviolet spectra of polypeptide films in various conformations. Figure 3 shows the far-ultraviolet spectra obtained from films cast from hot and cold water, respectively. Although quantitative comparisons are not possible for each isolated transition, comparison of the ratio of intensities for the n–π^* and π_1–π^* transitions would reveal if either of these bands were sensitive to conformation, unless each changed by the same amount. Table II summarizes the data obtained after resolving

Table II. Far-Ultraviolet Absorption Data for Poly(Ala-Gly-Gly)

	n–π^*		π–π^*	
	Wavelength	Extinction[a]	Wavelength	Extinction[a]
3_1	215	244	193	5250
Disordered	219	1045	197	5750

[a] Molar residue extinction.

these bands into component gaussians. It is evident that for the PGII conformation there is relatively little (4%) n–π^* component and the curve is almost entirely due to the π_1–π^* transition; however, the n–π^* transition at ~219 mμ was some 18% of the π_1–π^* transition for the disordered polypeptide: the low frequency plateau after the π_1–π^* band was accommodated by using a third gaussian, corresponding to the π_2–π^* transition, observed by other workers using vacuum ultraviolet spectroscopy[16] at ~170 mμ. Solution spectra were also run corresponding to these two conformations. The disordered spectrum was run upon quenching from 90° to 25° since circular dichroism spectra indicate that the disordered form did not reverse to the ordered form very rapidly, and this removes ambiguity associated with heating, due to solution expansion and the broadening of the water band below 185 mμ. The molar extinction coefficients for the π_1–π^* transitions are reported in Table II for the two conformations. These results are in agreement with the collagen–gelatin work and indicate that there is hyperchromism of both the π_1–π^* and the n–π^* transitions upon denaturation but that the effect is greater in the case of the n–π^* transition. The connection between these transitions observed with the absorption technique and the two bands seen in the circular dichroism spectrum is also apparent.

Infrared Spectroscopy. The position, relative intensity, and dichroism for the absorption bands seen in the infrared spectrum for an oriented film of poly(Ala-Gly-Gly) form I are shown in Table III. The amide A (a perturbed N–H stretching mode) is seen to be aligned parallel to the stroking direction; this is also thought to coincide with the long axis of the fibrous crystals seen in the electron microscope.[5] The splitting of the amide I into a weak band at ~1695 cm^{-1} and a major component at 1621 cm^{-1} with perpendicular and parallel dichroism, respectively, is indicative of an extended β structure with antiparallel peptide chains. This conformation is also suggested by the amide II at 1515 cm^{-1} and the amide V at 695 cm^{-1}. The dichroism for all these peptide vibrations suggests a cross β conformation since the molecular chain is found to be perpendicular to the stroking direction, *i.e.*, the fiber axis. The cross β structure has now been reported for a number of polytripeptides including poly(Ala-Glu(OEt)-Gly),[2] poly(Glu(OEt)-Glu(OEt)-Gly), poly(Glu(OEt)-Gly-Gly)[17] and poly(Ala-Ala-Gly)[18] under appropriate conditions. The identification of form I as a β structure is in agreement with previous work on this polymer; however, the band dichroism was not reported.

Table III. Principal Infrared Bands for the Three Forms of Poly(Ala-Gly-Gly)

Frequency, cm^{-1}				
Form I (β)	Dichroism	Form II	Form III	Assignment
3290	∥	3295	3293	Amide A
3070		3082	3072	Amide B
2929		2935	2931	CH_2 antisym str
1697	⊥	1656	1655	Amide I
1621	∥			
1515	⊥	1550	1535	Amide II
1435		1435	1420	CH_2 twist
1225		1245	1240	Amide III
690		735	730	Amide V
610		670	650	Amide VI ?

Infrared spectra were also taken from films obtained in the other two conformations. Dichroic spectra were

(15) W. B. Gratzer, W. Rhodes, and G. D. Fasman, *Biopolymers*, 1, 319 (1963).
(16) C. R. McMillin, W. B. Rippon, and A. G. Walton, *ibid.*, submitted for publication.
(17) J. M. Anderson, H. H. Chen, W. B. Rippon, and A. G. Walton, *ibid.*, in press.
(18) B. B. Doyle, W. Traub, G. P. Lorenzi, F. R. Brown, and E. R. Blout, *J. Mol. Biol.*, 51, 47 (1970).

taken for the ordered polyglycine II form; however, the amount of dichroism was less than that found for form I. This could be due either to poor crystallinity or orientation due to the higher concentration of polymer required for film-casting specimens for infrared spectroscopy coupled with the use of the relatively nonvolatile aqueous solvent; nevertheless, the amide A band appears to have parallel dichroism which would be consistent with the electron diffraction results reported elsewhere[5] and again suggests that the helix axis is perpendicular to the fiber axis. If infrared absorption bands shown in Table III for forms II and III are compared it is evident that there is very little shift in the amide A and amide I bands; however, the remaining bands are seen to have quite significant shifts confirming the presence of the different conformations seen with ultraviolet spectroscopy. The major changes are seen in the amide II and the amide B bands; the latter has previously been assigned to arise from Fermi resonance splitting of the NH stretching mode due to accidental degeneracy with the first overtone of the amide II mode. If this explanation is accepted then it is possible to follow the method outlined by Miyazawa[19] and calculate the frequency of the unperturbed N–H stretching mode. These calculations place the NH stretch at $\sim$3277 cm^{-1} for the ordered form and 3288 cm^{-1} for the random form. This increase in frequency along with the lowering of the amide II frequency would suggest that the hydrogen bonds between peptide groups are longer for the random form than the ordered form. Other smaller, but still significant, variations are seen in the modes associated with the methylene and methyl groups. These possibly reflect the changed environment of these groups due to the different conformations. Of particular interest, however, is the variation in the amide III region shown in Figure 4. Denaturation or melting of the helix results in a broadening of the amide III mode at 1245 cm^{-1} as well as a shift of some 5 cm^{-1} to the lower frequency. This broadening effect is so marked that a band at $\sim$1280 cm^{-1}, arising from a CH_2 wagging, possibly with some amide III component, is clearly resolved in the case of the ordered form but appears as an indistinct shoulder for the disordered form; this is in spite of the amide III band moving away to lower frequencies. The broader amide III is consistent with the less crystalline environment. Finally it should be pointed out that both the amide III and amide II modes contain contributions from C–N stretching and NH bending vibrations; however, the former is more dominant in the amide III mode while the latter predominates in the amide II mode. The results suggest that the perturbation in frequency of both these bands is due to the NH bending component more so than the C–N stretching mode—a conclusion consistent with the variation in hydrogen bonding suggested earlier.

Implications to Collagen Structure. The collagen triple helix may be considered to arise from the intertwining of three supercoiled polypeptide chains. These may be thought to exist in a distorted 3_1-helical conformation, a distortion which results in a reduction in the residue translation from *ca.* 3.1 Å to *ca.* 2.9 Å. Thus it is apparent that the form II of poly(Ala-Gly-Gly) described above may be considered a collagen model both

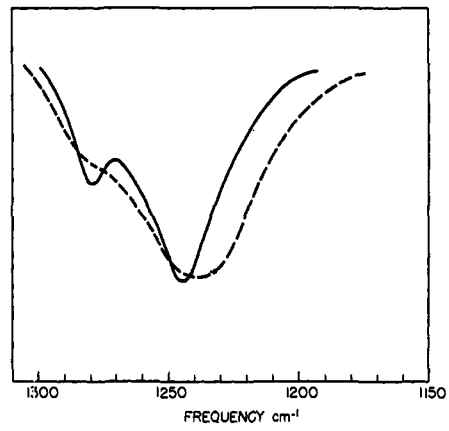


Figure 4. Amide III region of the infrared-absorption spectra for poly(Ala-Gly-Gly) in the disordered (- - -) and in the 3_1 helix (—).

in respect to sequence and conformation. However, the conformational similarity is crude and the high glycine content is not representative of the collagen sequence. In fact it will become evident that the stabilization of even this 3_1-helical form is related to the unusually high glycine content.

A number of glycine-containing polytripeptides, which omit proline residues, have now been studied and in all the reported cases a cross β conformation has been isolated. This prevalence of the β conformation, in part, reflects the stability of the intermolecularly hydrogen-bonded extended sheets. This conformation is not seen when proline-containing polytripeptides are studied due to the restricted rotations of the proline residue. On the other hand, poly(Ala-Gly-Gly) is the only such polymer to be completely characterized in what appears to be a pure polyglycine II conformation. One other such polytripeptide, poly(Glu(OEt)-Gly-Gly),[20] appears to have this 3_1-helical conformation in aqueous solution; however, at the moment, there is no solid state evidence for this conformation. Finally poly(Ala-Ala-Gly) has been reported to occur as a β sheet or an α helix along with what was considered to be a random form.[18] The latter, however, gave a circular dichroism spectrum at $-112°$ which was consistent with $\sim$75% disordered and $\sim$25% 3_1 helix. Such a mixture would give a circular dichroism spectrum corresponding to that of poly(Ala-Gly-Gly) at $\sim+60°$ if allowance is made for the additional center of optical activity in the disordered form of poly(Ala-Ala-Gly). Thus it now appears that polymers with two-thirds of their residues glycine are compatible with the 3_1-helical form and that the further substitution of one of those remaining glycines per tripeptide with amino acids containing β-carbon atoms destabilizes this helical form.

It is apparent, therefore, that the replacement of the proline residues in a polytripeptide with amino acids having greater rotational freedom will destabilize the supercoiled triple helical conformation. The latter form appears to require $\sim$two-thirds glycine for meaningful stability—a level which would not be expected to

(19) J. Miyazawa, *J. Mol. Spectrosc.*, 4, 168 (1960).

(20) W. B. Rippon and A. G. Walton, unpublished observation.

occur for many tripeptide units because of the overall amino acid content of collagen. Thus it is probable that isolated sequences within the collagen chain may not favor the triple helical structure, but rather, if this conformation persists it is likely to be the result of interactions with adjacent peptide sequences containing the "helix directing" proline residues.

It is of further interest to compare the conformations reported here for poly(Ala-Gly-Gly) with that reported for poly(Gly-Pro-Ala). The latter forms a triple helical structure which is stable at room temperature whereas the former forms a 3_1 helix as do the homopolymers of both glycine and proline. Initially it may appear that proline insertion has destabilized the PPII conformation; however, it is probably more correct to consider that the insertion of the proline residue has favored the specific aggregation of three chains to form a triple helix. In fact it is quite probable that poly(Ala-Gly-Gly) is aggregated in solution and that this aggregation is contributing to the stability of the 3_1-helical conformation.

Conclusions

Poly(Gly-Gly-Ala) has been obtained in the solid state, in three conformational forms, one of which is disordered. The form obtained is sensitive to the physical and chemical conditions of isolation. Each of these forms has been characterized using ultraviolet absorption and circular dichroism spectroscopy in conjunction with infrared spectroscopy. In addition there is evidence that one of these forms (3_1-helix form II) exists in dilute aqueous solutions of the polymer and has enabled the characterization of the optical properties for this biologically important conformation. Data are presented which indicate that there is a helix to disordered melting of the polymer in dilute aqueous solution with a melting temperature of *ca.* 45°.

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Helix–Coil Controversy for Polyamino Acids

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Abstract: A theoretical conformational analysis of the charged form of poly-L-glutamic acid in aqueous solution has been carried out. In accord with the proposal of Krimm, *et al.*, the most stable conformation is found to be an extended helix rather than a random coil as has been generally assumed. The conformational free energy and entropy have been calculated and free energy minimization procedures reveal that the helix parameters are $\phi = 84°$, $\psi = 321°$, $n = 2.4$ residues/turn, and $d = 3.2$-Å rise/residue. It now seems that the new conformation draws support from both experimental and theoretical investigations and may have important implications in the structural analysis of charged polypeptide chains, particularly in fibrous proteins.

A great deal of effort has been devoted to the study of the so-called helix–random coil transition of such poly-α-amino acids as poly-L-glutamic acid (PGA) and poly-L-lysine (PL). In fact this pH-induced conformational transition has been studied by most known physical methods.[1] It may seem strange, therefore, that the nature of the transition has only been seriously questioned in the past few years. There are two basic observations which lead to the belief that the charged form may not be random coil as previously supposed. First, the concept that a strongly charged polymer may be in a fully collapsed random form seems intuitively unlikely and may be checked by modern methods of conformational analysis. Secondly, spectropolarimetry, which is one of the more sensitive methods of conformational analysis, reveals that the spectrum of these charged species is distinctly different from the random secondary form of proteins, *e.g.*, gelatin. There are, though, aspects of CD–ORD spectra which need to be clarified before it can be stated that PGA, PL, etc., in their charged forms, are not disordered (but different from gelatin).

Most techniques applied to a study of the conformational transition are not capable of identifying conformations *per se*, this being particularly true of spectroscopic methods. Furthermore the techniques capable, in principle, of demonstrating the collapse of a rod-like (α helix) to a random coil sphere (*e.g.*, various light scattering methods) have not revealed the expected decrease in radius of gyration.[2]

The first experimental evidence of two different coil forms was presented by Tiffany and Krimm[3] who observed that the charged form of PGA in aqueous solution gave CD–ORD curves similar to those of poly-L-proline (PP) in the PPII conformation, but shifted to a lower wavelength. Treatment of the PGA solution with $LiClO_4$ changed the CD–ORD curves to the form normally associated with denatured proteins. It was also noted[4] that poly-L-proline in the PPII conforma-

(1) See for example, "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1969.

(2) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polym. Sci.*, 23, 851 (1957).
(3) M. L. Tiffany and S. Krimm, *Biopolymers*, 8, 347 (1969).
(4) M. L. Tiffany and S. Krimm, *ibid.*, 6, 1767 (1968).