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°.

Acknowledgment. The authors are pleased to acknowledge the research support of the National Institute of Dental Research under Program Project No. DE 02587. One of us (W. B. R.) gratefully acknowledges support through a National Institutes of Health Postdoctoral Fellowship.

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-

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tion also changes under the influence of concentrated $CaCl_2$ to a collapsed random form. Although there are similarities between the PGA and PPII systems in terms of the effect of concentrated electrolytes, the nature of the effect has been in some dispute. Various authors have suggested that divalent ions coordinate to the carbonyl in the peptide bond and consequently modify the nature of the active chromophore.[5,6] Most recently a study[7] of the Raman spectrum[8] of the collapsed form of poly-L-proline indicates that a mixture of cis and trans peptide bonds is present, seemingly providing support for the carbonyl coordination hypothesis. However, no similar evidence is available for PGA where it seems feasible that the negatively charged polyelectrolyte establishes a charged cloud of counterions including lithium. Whether or not this is due to intrinsic structural differences in amino and imino acids remains to be seen.

The disruption of the α helix of PGA and PL as the charged form develops suggests that the predominant conformation directing influence is the electrostatic repulsion between side chains. Krimm and Mark[9] have performed a simplified theoretical calculation in which the conformation of the charged polyamino acids is assumed to be dictated by electrostatic forces only. The lowest energy (vacuum) conformation was then found to be a left-handed helical conformation with approximately 2.5 residues/turn and a peptide repeat of *ca.* 3.4 Å. This conformation, being an extended helical form, may be regarded as a distorted left-handed polyglycine (or poly-L-proline II) helix and could apparently account for the general form of the circular dichroism curves for PGA and PL in the charged form. However at that time the precise nature of the CD curves for nonimino polypeptides in the polyglycine II form was unknown.

It has been pointed out[10,11] that although the water-soluble N-substituted esters of poly-L-glutamine have CD spectra similar to the charged forms of PGA and PL, the predominant conformational driving force in the former case cannot be electrostatic repulsion of the (uncharged) side chains.

In a further recent development, it has been shown[12,13] that poly(Ala-Gly-Gly) (PAGG), which forms optically active polyglycine II helices in aqueous (and other) solutions, has CD curves very similar (see Table II in ref 12) to those of charged PGA and PL, thus supporting the contention that the polyelectrolytes might have helical segments, probably left handed.

Thus, support for the hypothesis that charged PGA in aqueous solution assumes an ordered approximate PPII conformation draws support from: (1) the similarity of the PGA CD–ORD spectra to those of PPII,[2,4] and the similarity of the effect of counterions on these spectra; (2) the similarity of the PGA and PAGG[12,13] CD–ORD spectra; (3) the simplified energy calculations of Krimm and Mark.[9] In an attempt to extend this list we have conducted a complete theoretical analysis of the conformational free energy of PGA in aqueous solution.

Methods

The method used in this study to calculate the conformational energy of a polypeptide is based upon a semiempirical theory, portions of which were developed in this laboratory, which has been described in several previous publications.[14–19] In using this theory it is assumed that the conformational energy of a polymer in a solution (in this case aqueous solution) is the sum of the atomic pairwise interactions which include: nonbonded dispersion forces, torsional forces, electrostatic forces, polymer–solvent forces, and hydrogen-bonding forces. The free energy contributions from polymer–solvent interactions in aqueous solution were computed using a traditional hydration shell theory[20] modified by Gibson and Scheraga[21] and by Hopfinger.[22]

The peptide backbone geometry used is that suggested by Pauling and Corey.[23] A set of ideal bond lengths and bond angles, as listed by Dickerson and Geis,[24] was used to compute the side-chain atomic coordinates. The parameters used in the calculation of nonbonded dispersion interactions were those suggested by Ooi, *et al.*,[25] with the exception that the H–H interaction was assumed to be characterized by a 2.2-Å contact distance, rather than the earlier 2.4 Å. The charge distributions were calculated by the procedure advanced by Poland and Scheraga.[26] A dielectric of 3.5 was used throughout the calculations. A complete set of the interaction parameters may be found in ref 19.

The equivalence condition (*i.e.*, all analogous dihedral angles in different residues are equal) was applied throughout the calculations on neutral and ionized PGA. All calculations were made on a chain of nine residues. This length was found to be sufficient to simulate the infinite chain.

The stable backbone conformations were located by conducting a systematic scan of ϕ, ψ space and isolating broad regions where minima had to exist. The minima were then located more precisely by employing a procedure which minimized the conformational potential energy as a function of all backbone and side-

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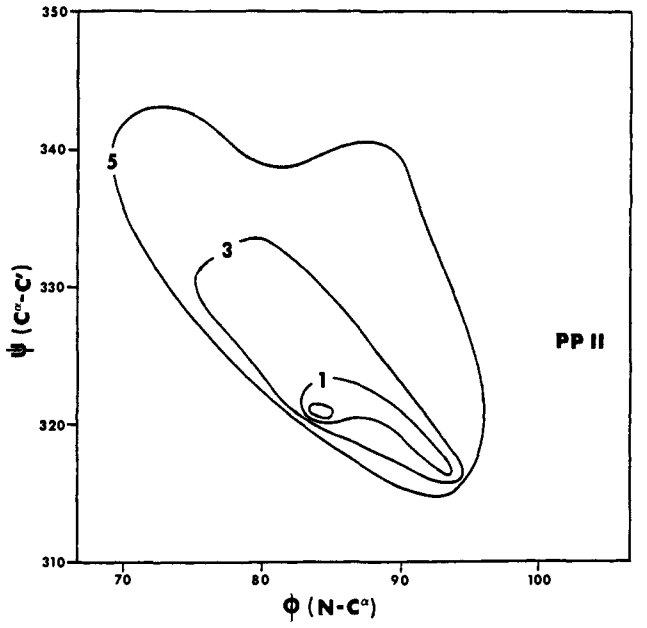

Figure 1. Relative energy contours in kcal/mole-residue for ionized PGA in aqueous solution, extended helix conformation. Relative energy of minimum at (84°,321°) is 0.0 kcal/mole-residue. Position of PPII helix is indicated.

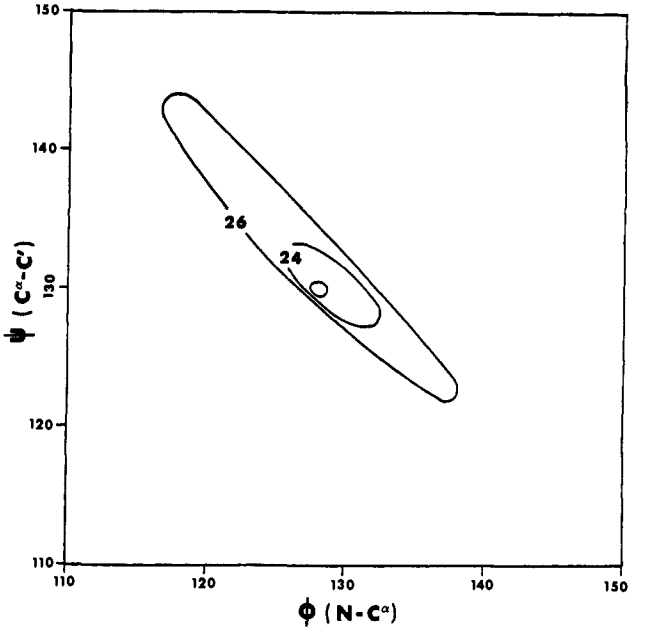

Figure 2. Relative energy contours in kcal/mole-residue for ionized PGA in aqueous solution, α-helix conformation. Relative energy of minimum at (128°,130°) is 21.6 kcal/mole-residue.

chain dihedral angles. The procedure seeks out a minimum by sampling conformational hyperspace with the constraint that the new point being sampled is arrived at by a random perturbation around the position which yielded the lowest energy found in the scan up to that point.

An infinite, isotactic homopolymer in a homogeneous solvent will obey the equivalence condition. As Huggins has established,[27] once the homopolymer is in a local minimum any small perturbation of one residue will result in a potential gradient tending to restore the residues to complete equivalence. It is only when a statistical perturbation is applied to the residues that their environments are no longer identical and the equivalence condition may be relaxed. Such, apparently, is the effect of counterions on the ionized chain. We have attempted to model this perturbation by considering the completely ionized chain with counterions coordinated to randomly selected side chains. The stable conformations of this chain were located by the random minimization procedure, using the minima of the fully ionized polypeptide as starting points. In this calculation the equivalence condition was completely relaxed to assume no *a priori* correlation between residues.

The conformational energy was calculated at a sufficiently large number of random points in the vicinity of each minimum to justify an estimation of the conformational entropy. The unnormalized statistical weight of the jth point, p_j, was computed by $p_j = \exp(-\Delta E_j/RT)$, where ΔE_j is the energy difference between the jth conformational data point and the minimum. The set of statistical weights was normalized, such that: $\Sigma_j p_j = 1$. The entropy, S, associated with the minimum is then given by the Boltzmann relation: $S = -R\Sigma_j p_j \ln p_j$. The entropy calculation on the α helix included 47 data points, and that on the extended conformation included over 200. The neighborhood of each minimum generally included all conformational hyperspace within ±20° of each degree of freedom.

The conventions of Edsall, *et al.*,[28] are used throughout this paper.

Results and Discussion

An exhaustive search of the upper left-hand quadrant of conformational space yielded a global minimum at $(\phi,\psi) = (84°,321°)$ for ionized PGA. This left-handed helix has 2.4 residues per turn and a 3.2-Å rise per residue. The corresponding values for the PPII helix at (103°,326°) are 3.0 residues per turn and 3.1-Å rise per residue. The relative conformational energies and the entropies *in vacuo* and in aqueous solution for the extended coil and the α helix are given in Table I.

Table I. Conformational Energies and Entropies of Ionized PGA

Conformation		U, kcal/mole-residue	S, eu/mole-residue	$-TS$,[a] kcal/mole-residue
Right-handed α helix	Vacuo	+46.8	5.6	−1.7
	H_2O	+21.6	6.7	−2.0
Extended helix	Vacuo	+31.0	7.3	−2.2
	H_2O	0.0	6.9	−2.1

[a] T = 300°K.

The shape of the extended coil minimum is shown in Figure 1. The α helix minimum is shown in Figure 2, for purposes of comparison. It will be noted that the extended helix has a somewhat broader minimum than the α helix but should be considered a definite conformation.

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Table II. Conformational Energies of Neutral PGA

Conformation		U, kcal/mole-residue
Right-handed α helix	Vacuo	+14.0
	H_2O	+1.6
Extended helix	Vacuo	+15.8
	H_2O	0.0

As a check on the calculations the relative conformational energies of neutral PGA are given in Table II. These show the α helix to be the more stable conformation *in vacuo*.

Thus, the contention that ionized PGA assumes an ordered conformation in aqueous solution gains further support from this work.

To reproduce the effect of $LiClO_4$ on the conformation of ionized PGA positive counterions were coordinated to randomly selected side chains. Minimization of the conformational potential energy indicated that disruption of interresidue order resulted in a more stable conformation than did maintenance of the equivalence condition. Although the computer time needed to determine its exact location would be prohibitive, it seems clear that a global minimum should exist for any particular configuration of residues and counterions. However, over a large number of residues the random coordination of counterions would lead one to expect a random distribution of dihedral angles, especially when the kinetic aspects of coordination are considered.

These calculations indicate that the order–disorder transition of PGA is that associated with the addition of counterions, not with the initial ionization of the side chains. Thus, it appears that the experimental effect may be modelled by theoretical methods without the need of assuming a bond with carbonyl group. One might suppose that similar effects may be observed with other charged polypeptides and proteins.

The greatest anomaly in the experimental data relevant to this discussion is the result of Adler, *et al.*,[10] that poly(N^5-(2-hydroxyethyl)-L-glutamine) (PHEG) in its non-α-helical conformation has CD–ORD spectra similar to those of ionized PGA, although its side chains are uncharged. There is at present no adequate explanation for this result. However, we wish to point out two features of the PHEG CD spectrum in which it differs from that of PGA: (1) the molar ellipticities of PHEG are half those of PGA, suggesting that the former has less conformational integrity; (2) the ellipticity of the 235-mμ band relative to that of the 217-mμ band is a factor of ten greater for PHEG than for PGA. The significance of the 235-mμ band is presently unclear. Myer has suggested[29] that it arises from a two-state mixture of extended helix and α helix. Experimental work being conducted in this laboratory[30] indicates that the band arises when the extended helix conformation is "loosened," *i.e.*, when each residue is energetically able to occupy a larger volume of conformational hyperspace. Such would be the case for PHEG, where the lack of electrostatic side chain–side chain interactions would presumably decrease the energy gradients experienced by the backbone. Tiffany and Krimm[3] were unable to detect the 235-mμ band in the high pH PGA spectrum at very low salt concentrations, but found that the band could be introduced by lowering the pH or by increasing the salt concentration, *i.e.*, by decreasing the electrostatic interactions of the side chains. Thus, although the PHEG spectra are anomalous, they should not yet be taken as contradictory to any results reported here.

It should be pointed out that the "persistence lengths" mentioned by Krimm and Mark[9] are of little significance in discussions of the completely ionized polypeptide. The above mentioned homogeneity of the calculations precludes any deviation from the equivalence condition, which would be implicit in any model of the chain consisting of extended helical segments connected by short random segments. The concept of "persistence lengths" apparently arose from an attempt to model the long polypeptide chain with short oligomers—a method which ignores many important interactions.

Several workers have attempted to predict the optical properties of the unordered or random chain. Aebersold and Pysh[31] were unable to reproduce the features of the CD spectrum when either a randomly generated coil or the extended helix was used as a model. This failure could originate in the inadequacies of the models or from the shortcomings of their optical theory. There is some indication[32] that a randomly generated coil can act effectively as the model for calculation of the unordered CD spectrum, as observed in heat-denatured collagen and PGA at high salt concentration.[3] A recent study by Zubkov, *et al.*,[33] suggests that the CD spectrum predicted for the extended helix is similar to that observed for PGA at high pH.[3]

The present calculations may be of some significance when it is realized that many fibrous proteins possess regions which are rich in polar residues but are not random in conformation.[34] The structure of sequential polypeptides containing charged residues will be the subject of a future communication.

Terminology for the new conformation has not yet been considered in detail—two main possibilities present themselves: the "charged coil" (maintaining the helix–coil concept) or the "extended helix" (being somewhat more descriptive).

In conclusion, we may state that available evidence, along with the conformational analysis presented above, affirms the stability of the left-handed extended helix conformation for charged polypeptides, poly-L-glutamic acid in particular.

Acknowledgments. We are pleased to acknowledge the financial support of the National Institute of Dental Research under Program Project No. DE 02587.

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