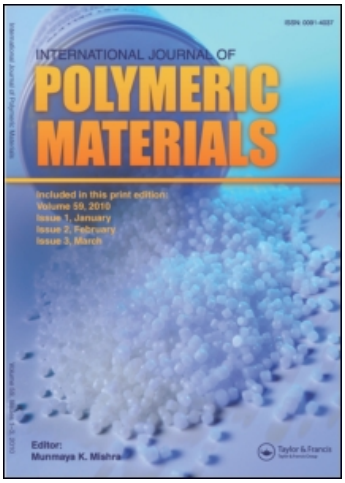

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The 3_1 Helix-Coil Transition for Polypeptides. Circular Dichroism Studies†

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Circular dichroism has become a popular method for following conformational transitions induced in optically active polymers. Recent refinement of experimental spectra obtained from model polypeptides by computer fitting to spectra obtained from solutions of proteins of known conformation has verified the applicability of the models chosen for the α helical and β conformations. However, the spectrum required for disordered regions was in conflict with much of the literature and agrees with our assignment based on studies of collagen and collagen models at elevated temperature. This spectrum consists of two troughs, one at $\sim$ 225 nm and the other at $\sim$ 200 nm. The latter had previously been associated with random polypeptides and we have shown the former to be sensitive to disorder in polymers approaching the 3_1 helical conformation. This paper presents the results from a study of three polypeptides which undergo a 3_1 helix $\rightarrow$ disorder transition.

1 INTRODUCTION

The most prevalent mammalian protein, collagen, has as its basic structural unit the tropocollagen molecule. This molecule has been shown to consist of three polypeptide chains wound around each other in a rope-like fashion. The conformation of these three chains may be considered to arise from a tightening of a 3_1 helix with a 3.1Å peptide repeat down to a 10_3 helix with a 2.86Å peptide repeat.[1] This also results in the formation of a right handed superhelix in each chain. A considerable amount of work has been directed to studying this protein and its thermal denaturation to gelation. Solution techniques have

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included viscosity,[2] sedimentation equilibrium,[3] gel permeation chromatography,[4] ultraviolet absorption spectroscopy,[5] and circular dichroism spectroscopy.[6] The first three of these methods depend on changes in hydrodynamic volume associated with the unravelling of the three chains whereas the last two, potentially, are capable of following loss of the 3_1 helical structure with or without any change in molecular weight. Circular dichroism, however, is not an absolute method for structural determination and thus, relies on "calibration" from polypeptides of known conformation.

Unfortunately, in the past the only optically active polymer with the 3_1 helical structure was polyproline and although a partial disruption of this molecule resulted from heating, precipitation occurred before total disruption could be achieved.[7] In addition, the chromophore studied in the ultraviolet region was different, due to the imino acid repeat unit, to that found in poly (α-amino acids). Thus there were no satisfactory models to use for elucidation of certain features of the collagen → gelatin transition. Recently we have synthesized and studied poly(Ala-Gly-Gly) which was shown to undergo a 3_1 helix → disorder transition,[8] a transition which has now been followed using X-ray diffraction,[9] infrared spectroscopy, ultraviolet absorption and circular dichroism spectroscopy.[10]

Identification of the circular dichroism spectrum with a positive peak at ~ 215 nm and a trough at ~ 190 nm with that of a 3_1 helix, and a spectrum with a small trough at ~ 225 nm and a larger trough at ~ 200 nm with that of a random polypeptide—although consistent with collagen and gelatin spectra—was not in accord with the accepted model for random polypeptides, *i.e.*, poly(L-glutamic acid) or poly(L-lysine).[11] These charged polymers gave circular dichroism spectra similar to that of poly(Ala-Gly-Gly) in a 3_1 helix. Independently other workers have concluded that they were not random but rather had a sufficiently "recurrent pattern of replication of bond conformations" which approached those of a 3_1 helix. The terminology *extended helix* was adopted.[12–14] There now appear to be two major schools of thought. On the one hand it is suggested that polyelectrolytes are still satisfactory models for random regions of proteins, and possess no significant ordering of adjacent units, whereas, others feel that such polyelectrolytes possess ordering of adjacent units approaching that of a 3_1 helix, with considerable flexibility in the chain. The controversy was recently highlighted in a popular way by the molecular biology correspondent of *Nature*. Evidence for the former view comes mainly from viscosity data, however it should be pointed out that such hydrodynamic studies were unable to distinguish between poly(L-proline), I and II, forms[15] which give vastly different CD, X-ray, infrared,[7] and Raman spectra.[16] Evidence for the latter view relies heavily on circular dichroism data with support from conformational calculations which demonstrate the feasibility of this assignment.

Earlier this year we reported the results of a study on a polydipeptide with alternate charged residues which also appeared to adopt this $\sim 3_1$ structure in solution.[17] A further study of this and other polypeptides reported in the literature, suggested that the trough at $\sim$ 225 nm in the random form, which had previously been assigned to residual α helix, was in fact characteristic of random polypeptides. The 3_1 helix thus resulted in a positive band $\sim$ 215 nm and a negative band at $\sim$ 190 nm with a small negative band seen when the 3_1 helix was less well developed. These were assigned to $(+)\ \pi - \pi^*$, $(-)\ \pi - \pi^*$ and $n - \pi^*$ transitions. Disorder resulted in loss of splitting of the $\pi - \pi^*$ to give a single band at $\sim$ 200 nm and a trough, due to the $n - \pi^*$ transition at $\sim$ 225 nm.[18] This interpretation of the data was made more plausible by results published for computer fitting of circular dichroism curves for α, β, and random forms to obtain consistency between circular dichroism and X-ray data for a number of proteins.[19] The results for the α and β forms were consistent with those already obtained from model studies.

In this study we wish to report circular dichroism data for a series of polypeptides which we believe undergo a 3_1 helix $\rightarrow$ disordered form transition. The transition can be induced by heat or in one instance addition of electrolyte.

2 MATERIALS AND METHODS

Poly(L-lysine.HCl) was obtained from Miles Laboratory and had a reported molecular weight of 70,000. The synthesis and characterization of poly (Ala-Gly-Gly) has been described previously.[10,20] Poly(Glu-Ala) was kindly supplied by Dr. Kovacs of St. Johns University and has been previously characterized.[17] Circular dichroism measurements utilized a JASCO J20 recording spectropolarimeter. Quartz cells with water jackets maintained at constant temperature by circulation of water from a Nestlab TE3 water bath were used for all experiments. Since poly(L-lysine) is sensitive to pH and salt concentration the same solution was used for observing the $\sim$ 225 nm trough and the $\sim$ 215 nm peak by utilizing cells of 10 cm and 0.5 cm path lengths. The solutions of the charged polymers were at pH 6.

3 RESULTS AND DISCUSSION

Figure 1 shows circular dichroism spectra for poly(Glu-Ala) in a mixed 3_1 helical form and random form. This spectra illustrates the bands typical of those obtained for the series of polypeptides discussed below and they have tentatively been assigned as indicated.

Figure 2 shows a plot of the ellipticity of the positive $\pi - \pi^*$ component

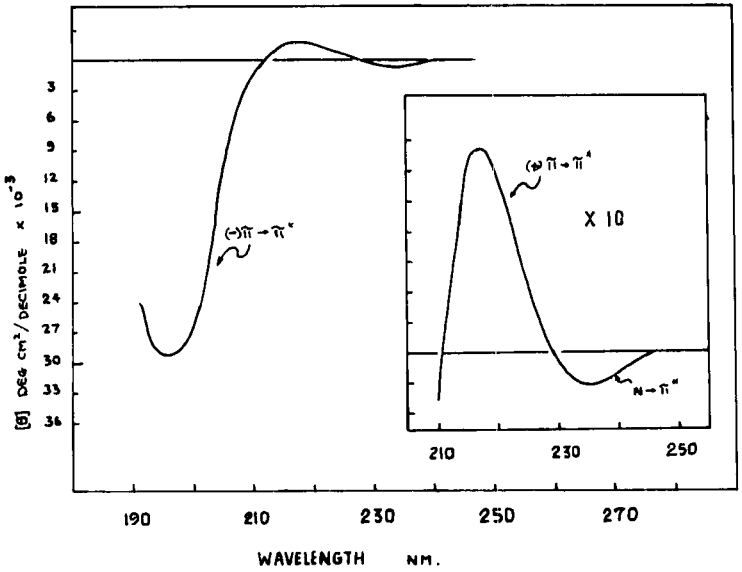

FIGURE 1 Circular dichroism spectrum of poly(Glu-Ala) at pH 6 and 25°C, showing the three bands which are studied below.

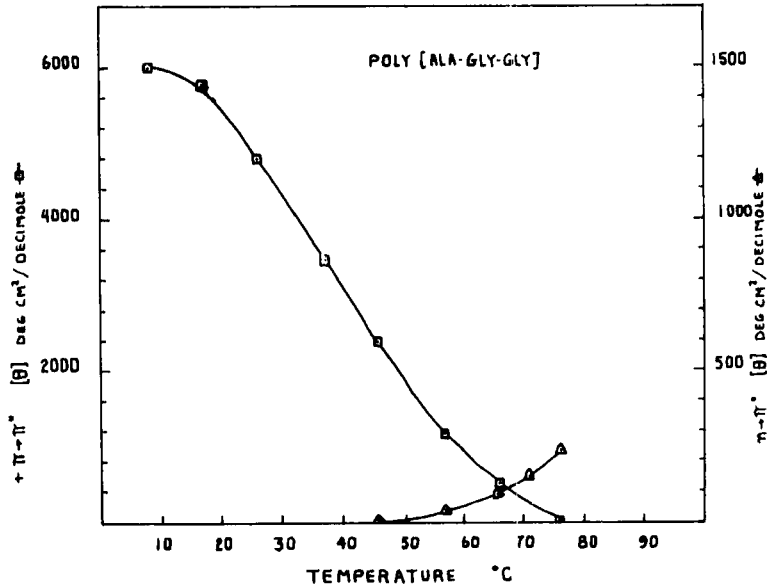

FIGURE 2 Plot of ellipticity of the (+) $\pi \rightarrow \pi^*$ (□) and $n \rightarrow \pi^*$ (△) transition of poly(Ala-Gly-Gly) as a function of temperature at neutral pH.

and the $n \rightarrow \pi^*$ ellipticity versus temperature for poly(Ala-Gly-Gly). Similar plots for poly(Glu-Ala) and poly(L-lysine) are shown in Figure 3. Comparison of these plots shows that they fall into two classes. On the one hand the $(+)\ \pi \rightarrow \pi^*$ data for poly (Ala-Gly-Gly) is concave down at the lower temperatures, approximately linear for intermediate temperatures and concave up at higher temperatures whereas similar data for the other two polymers is essentially linear. This difference may reflect the different nature of the stabilizing forces which in the first case arise presumably from peptide bond carbonyl solvation as is the case with polyproline II, and possibly some polymer/polymer packing of short range, due to the limited solubility, similar to that observed in the solid state.[9] On the other hand this would be unlikely for either of the other two polymers because of the side chain bulk and electrostatic repulsions.

Comparison of the curves for the charged polymers (Figure 3) indicates that the poly(L-lysine) data is very similar to that obtained for the poly(Glu-Ala) but shifted some 25°C higher—thus the helix stabilization appears to involve similar but weaker forces. This shift in stability is evident from both the $(+)\ \pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ data, and possibly reflects the role of electrostatic repulsion between side chain charges in stabilization. In the homopolymers

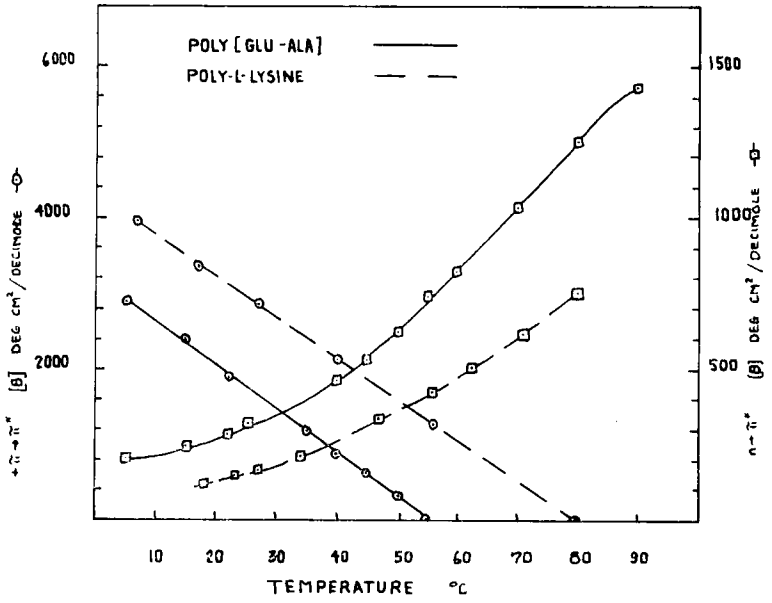

FIGURE 3 Plot of the ellipticity of the $(+)\ \pi \rightarrow \pi^*$ (○) and $n \rightarrow \pi^*$ (□) transitions of poly(L-lysine) (—— ——) and poly (Glu-Ala) (———) as a function of temperature at pH 6.0.

this would be a greater force of stabilization since 3_1 helix results in staggering the charges by 120°, whereas the polydipeptide has only alternating charges and thus more conformational space available. Backbone solvation could again feature in both of these cases.

If the ellipticity of the $n - \pi^*$ transitions, when the $\pi - \pi^*$ transition shows no positive component, are compared it is found to be $\sim$ 750 deg cm^2/dmol for both poly(L-lysine) and poly(Glu-Ala) whereas it is only 239 deg cm^2/dmol for poly (Ala-Gly-Gly). This difference reflects the symmetry of the glycine residues which in the absence of helical assymetry show no circular dichroism. Thus, in the disordered form only alanyl residues contribute to optical activity. This also results in a reduction of the $\pi - \pi^*$ transition for disordered poly(Ala-Gly-Gly) compared with the other polymers.

Figure 4 shows the changes in ellipticity for the $n - \pi^*$ and $(+)\ \pi - \pi^*$ transitions of poly(Glu-Ala) as a function of ionic strength. It is evident that there are considerable changes in the ellipticity of both bands similar to those induced by heat. However, when the $(+)\ \pi - \pi^*$ transition is zero the $n - \pi^*$ transition now shows an ellipticity of 1200 deg cm^2/dmol and in general the $n - \pi^*$ transition is more intense for a given value of the $(+)\ \pi - \pi^*$ component. This possibly reflects the different mode of helix disruption which now

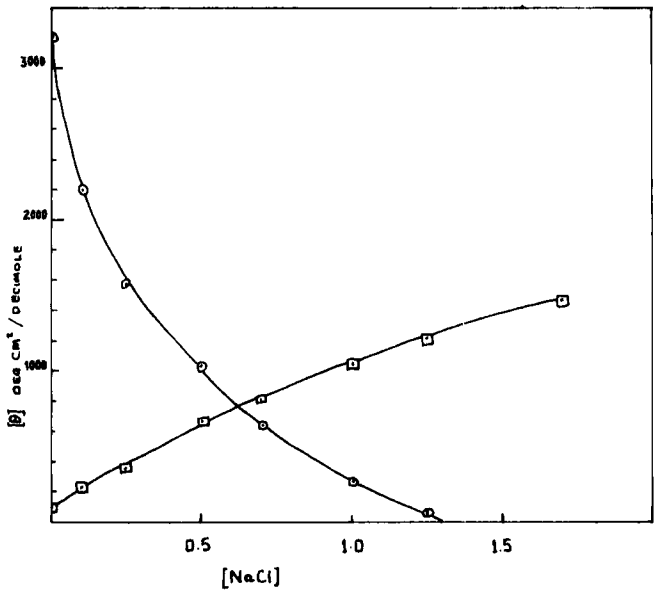

FIGURE 4 Plot of the ellipticity of the $(+)\ \pi - \pi^*$ (○) and the $n - \pi^*$ (□) transition of poly(Glu-Ala) as a function of electrolyte concentration at pH 6.0 and 25°C.

involves reduction of the electrostatic interaction and opens up a different region of conformational space than heating the polymer.

The wavelength for the minima due to the $n \rightarrow \pi^*$ transition is dependent on the relative magnitude of the $n \rightarrow \pi^*$ transition to the (+) $\pi \rightarrow \pi^*$ transition since they are only 10 nm apart, and hence overlap (width at half height being $\sim$ 30 nm). This makes the wavelength of the $n \rightarrow \pi^*$ transition appear to shift dramatically with increasing disorder—however preliminary curve resolving suggests that the curves can be accommodated with a constant position for both transitions. However, comparison of the wavelength of $n \rightarrow \pi^*$ transitions for heat treated and salt treated poly(Glu-Ala) indicates that the transition is some 3–4 nm lower in 0.1 *M* NaCl than in deionized water. This would be consistent with the behaviour of $n \rightarrow \pi^*$ transitions as solvent polarity is increased.[21]

Work currently in progress involves a study of proline containing synthetic polypeptides which can undergo the 3_1 helix $\rightarrow$ disordered transition. Preliminary work indicates that the $n \rightarrow \pi^*$ transition for proline is about one-fifth that of amino acids and occurs at $\sim$ 235 nm. Extension of this type of study to a cyanogen bromide fraction of collagen and finally collagen itself should enable us to distinguish (1) if chain loosening or premelting of the individual chains of tropocollagen occurs prior to strand separation and (2) which regions of collagen—proline rich or, as model studies would suggest, proline sparse—succumb to disruption first. It should be mentioned that there is already evidence that in reformation of collagen triple helices chain ordering can precede aggregation.[22]

4 CONCLUSIONS

The melting or disruption of three polypeptides from their 3_1 helical conformation has been followed using circular dichroism. Evidence from the temperature behaviour of bands assigned to the positive component of the $\pi \rightarrow \pi^*$ transition and the $n \rightarrow \pi^*$ transition suggests that the forces of stabilization for poly(L-lysine) and poly(Glu-Ala) are similar to each other and different to those of poly(Ala-Gly-Gly). Further, the salt denaturation of poly(Glu-Ala) possibly involves a different conformational pathway to the heat denaturation. Changes in the $n \rightarrow \pi^*$ transition frequency in the presence of electrolyte are consistent with the $n \rightarrow \pi^*$ assignment.

Acknowledgement

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DISCUSSION

Prof. D. Poland (*John Hopkins University, Baltimore, Maryland*): How does titration affect the amount of 3_1 conformation in polyglutamic acid?

Prof. W. B. Rippon: At low pH the 3_1 conformation is maximal, with lower temperatures also favoring this conformation. Increasing the pH and neutralization of side chains results in reduced 3_1 content. There is, however, controversy over the nature of the other conformational species. One suggestion is that small amounts of α helix exist, thus making a 2-state transformation from left-handed 3_1 helix to right-handed α helix. I feel, however, that a more satisfactory explanation is an intermediate randomization or loosening of 3_1 helix followed by formation of α helix when the titration is continued. The evidence for this is twofold. (1) Poly(Ala-Gly-Gly) CD curves show the same progression with temperature and α helix is most unlikely due to high glycine content. Therefore we do not need to propose α helix for explanation. (2) Poly (Glu-Ala) undergoes a 3_1 helix $\rightarrow$ α helix transition but temperature affects the bands from the partial 3_1 helix in the opposite way to bands from the α helix. Thus 3_1 helix $-$ α helix mixture is not compatible. (See Refs. 17 and 18.)