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- (79) It has been suggested by the referee that the degree of flexibility at the Gly CH₂ carbon appears reduced in cyclo(L-Leu-Gly) and cyclo(L-Phe-Gly) relative to the other two Gly-containing diketopiperazines (assuming the same orientation factors hold). The Phe ring seems to have internal motion about the C_β-C₁ bond as judged by the different *NT₁* values for C-4 and C-2,3; likewise, the Leu methyls appear to be free to rotate. Given this situation, it may be that the angular range (which clearly is in the flagpole-boat conformation is subject to some internal motion that allows it to sweep out a large solid angle above the diketopiperazine ring.
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Vacuum Ultraviolet Circular Dichroism of Poly(L-proline) I and II¹

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Abstract: The vacuum ultraviolet circular dichroism of helical poly(L-proline) I and II was measured. The circular dichroism of poly(L-proline) II in trifluoroethanol was measured to 162 nm; the ellipticity is negative below 220 nm, indicating that exciton interactions are not the dominant source of circular dichroism for the π - π^* transition in this conformation. The circular dichroism of a film of poly(L-proline) II cast from aqueous solution was measured to 135 nm. In the vacuum ultraviolet region two strong negative circular dichroism bands were observed at 146 and 178 nm, and the positive band near 230 nm was observed to be more intense than it is in solution. The observation of negative ellipticity between 135 and 230 nm indicates that the π - π^* transition must be strongly coupled to transitions below 135 nm. The interconversion of poly(L-proline) I \rightarrow II was studied by casting a series of films from trifluoroethanol solution 3 min, 88 min, 179 min, 291 min, and 52 hr after preparation of the solutions and measuring the circular dichroism of each film to 135 nm. A negative band was observed in poly(L-proline) I (3 min film) at 150 nm and a broad negative plateau between 165 and 175 nm indicates the presence of another band in that region. A treatment of the spectra depicting the interconversion process (poly(L-proline) I \rightarrow II in trifluoroethanol) yields a first-order rate constant of $7.8 \times 10^{-3} \text{ min}^{-1}$ ($t^{1/2} = 89 \text{ min}$) in agreement with previous workers.

The vacuum ultraviolet circular dichroism spectra of α helical poly(γ -methyl L-glutamate) and poly(L-alanine) have been reported,^{3,4} the latter from this laboratory. In this paper we report the vacuum ultraviolet circular dichroism (VUCD) of the helical conformations of poly(L-proline) I and II (PPI, PPII). In the near-ultraviolet (180–240 nm) circular dichroism (CD) of PPI a pair of oppositely signed bands are observed within the region of the π - π^* transition.^{5–7} These have been understood as arising largely from exciton interactions, and calculations of the exciton contribution to π - π^* CD in this conformation are in qualitative agreement with experiment.^{8–10} On the other hand, the near-ultraviolet CD of PPII is predominantly negative,^{5–7} indicating either that exciton interactions are not dominant in this loosely wound helix^{8–10} or that there is a positive exciton component CD band outside the range of measurement accessible with commercial instruments.¹¹ One purpose of this work is to show that the first of these two descriptions is the correct one. In the course of doing so, we have observed two new optically active transitions in the vacuum ultraviolet region for both PPI and PPII and have studied the interconversion of PPI to PPII in trifluoroethanol.

Experimental Section

Our instrument is the same as that used in our earlier work on poly(L-alanine)^{3,12} with the exception that here we used an improved light source.¹³ With this instrument the low wavelength limit is determined, in the case of solution studies, by solvent absorption and, in the case of film studies, by the combined low transmittance of the CaF₂ optical elements and of the polymer

film itself. All spectra reported here were taken with a spectral slit width of 1.66 nm, a time constant of 10 sec, and a scan rate of 2 nm/min.

Poly(L-proline) obtained from Miles Laboratories, Elkhart, Ind. (Lot PR-17.MW 6730) was in form I by CD criteria.^{5,6} After several days in trifluoroethanol solution conversion to form II is complete.⁵ Our solution cell consisted of two CaF₂ disks separated by aluminum foil spacers. The thin layer of solution was protected from evaporation by sealing the edges with O-rings under light pressure. With such a cell we were able to obtain spectra to 162 nm, below which absorption by the solvent became excessive.

To prepare films of PPII from aqueous solution, PPI as purchased was suspended in water and periodically shaken for several days. Films cast from the clear filtrate displayed the near-ultraviolet CD characteristic of form II.^{5,6} With our instrument we are able to obtain VUCD spectra of films 1000–3000 Å thick to 135 nm routinely and occasionally to 127 nm. It is known that the circular dichroism of polymer films can show birefringence effects, in that the signal obtained with such films depends upon the orientation of the film in the light path. If we observed such an orientational dependence with a film, that sample was discarded.

For the time study of the poly(L-proline) I \rightarrow II interconversion process,⁵ 20 mg of PPI as purchased was suspended in 4 ml of trifluoroethanol at 25°. With this procedure dissolution was complete in 4 min, which allowed us to define a t_0 for the interconversion process as being 2 min after addition of the polymer, and having an uncertainty of ± 2 min. Small samples of this solution were withdrawn periodically and placed onto 1-mm thick CaF₂ disks in a nitrogen filled glove box. Evaporation of the solvent was complete within 1 min, which allowed us to define a t_f as the time at which solvent evaporation was complete and having an uncertainty of ± 1 min. We adopted $t = t_f - t_0$ as the time available to the polymer for mutarotation.

Each film was further dried under a stream of dry nitrogen and

then in the vacuum chamber of the spectrometer. Films prepared this way were stable for extended periods, as judged by the constancy of the VUCD obtained over a period of time. Several films were cast at each time interval and one was selected on the basis of the insensitivity of its spectrum to rotation about the beam axis. For each film the absorbance maximum near 205 nm was determined to be less than 1, and the absorbance at 135 nm was estimated to be approximately 1.5. For purposes of presentation the VUCD spectra of the films were then scaled to constant absorbance.

Results

The VUCD of poly(L-proline) II in trifluoroethanol is shown in Figure 1. A small positive band is located near 227 nm and a large negative band is located at 206 nm; these two bands are within the range of commercial CD spectrometers.⁵⁻⁷ In our spectrum the maximum negative ellipticity is 17 times greater than the maximum ellipticity of the weak positive band, and the crossover between these two bands is near 223 nm. The ellipticity decreases from its maximum negative value at 206 nm to 182 nm beyond which the ellipticity once again becomes more negative. There is another apparent extremum at 167 nm beyond which the ellipticity decreases in magnitude to 162 nm, at which point solvent absorption causes excessive noise in the signal. The noise in the signal at wavelengths greater than 167 nm is less than $\pm 4 \times 10^{-3}$ deg; and from 167 to 162 nm the noise increases to $\pm 6 \times 10^{-3}$ deg. Therefore, the existence of a negative band at 167 nm cannot be established from the data in Figure 1 alone.

The VUCD of a poly(L-proline) II film cast from water is shown in Figure 2. The positive band at 230 nm and the stronger negative band at 214 nm are in the range available to commercial CD spectrometers.⁵⁻⁷ In this film spectrum the maximum ellipticity of the negative band is four times greater than the maximum ellipticity of the positive band, and the crossover between the two bands is at 223 nm. Below the large negative band at 214 nm the ellipticity decreases in magnitude to 192 nm below which it becomes more negative until a resolved band appears at 178 nm. Below this band the ellipticity decreases to 162 nm, followed by another well-resolved negative band at 146 nm. The ellipticity then becomes smaller until the noise-determined cutoff is reached at 134 nm. Above 145 nm the noise in the signal is less than $\pm 2 \times 10^{-3}$ deg and from 145 to 134 nm the noise increases to $\pm 4 \times 10^{-3}$ deg. Therefore, the existence of two previously unreported negative bands in the vacuum ultraviolet region is well established by the data of Figure 2. The intensities of these two bands are approximately equal, being about half that of the negative band at 214 nm.

Figure 3 shows the results of our time study of the interconversion process PPI \rightarrow PPII. The 3-min spectrum (curve 1) shows a large positive band at 216 nm and a crossover at 204 nm, below which the ellipticity remains negative to 135 nm. The spectra obtained at later times show the positive band decreasing until at 52 hr a large negative band appears at 214 nm. At the same time, the ellipticity in the vacuum ultraviolet region remains negative with well-resolved bands appearing at 146 and 178 nm.

Discussion

Our spectrum of poly(L-proline) II in trifluoroethanol shown in Figure 1 is essentially identical with the one reported by Bovey and Hood⁵ in the region from 190 to 240 nm. The small positive band at 226-227 nm is followed by a stronger negative band at 206 nm. The crossover between these bands is near 223 nm, and the ratio of their magnitudes is 17. Between 190 and 185 nm the ellipticity reported

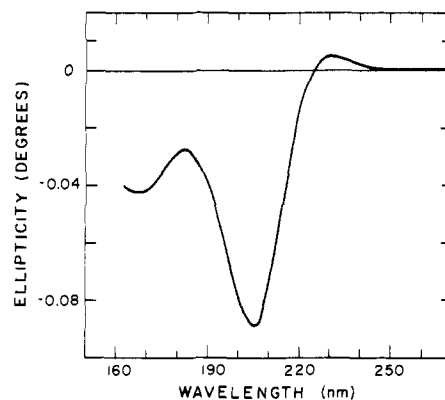


Figure 1. Circular dichroism of poly(L-proline) in trifluoroethanol (PPII).

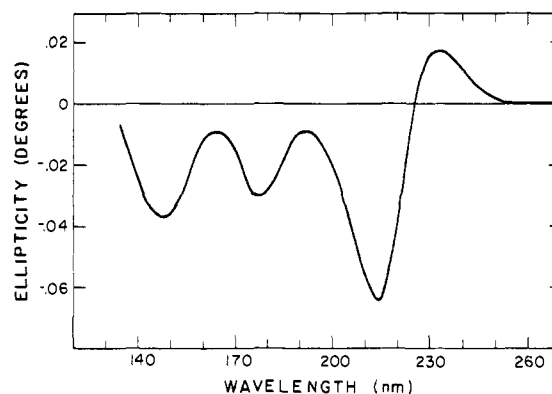


Figure 2. Circular dichroism of a poly(L-proline) film cast from aqueous solution (PPII).

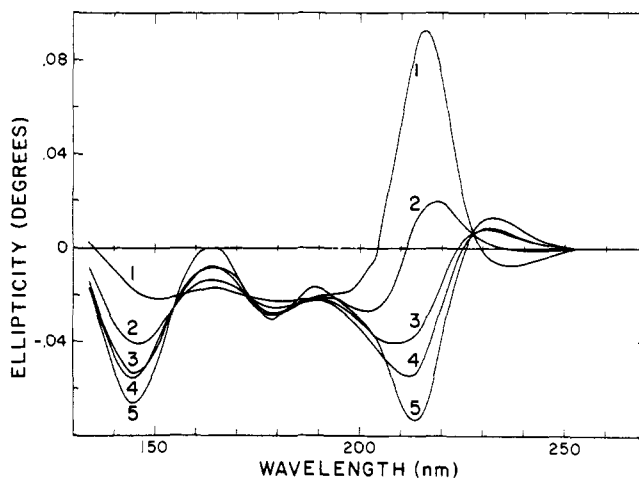


Figure 3. Circular dichroism of poly(L-proline) films cast from trifluoroethanol: (1) 3 min, (2) 88 min, (3) 179 min, (4) 291 min, (5) 52 hr.

by Bovey and Hood approaches zero,⁵ but we feel that since 185 nm is the limit of the instrument they used, our spectrum is more accurate. The ellipticity drops to one-third its maximum negative value at 206 nm, until a turning point is reached at 182 nm, outside the range of commercial CD instruments. While an additional negative band at 167 nm cannot be firmly established from the spectrum shown in Figure 1, because of substantial noise in the signal in that region, the appearance of additional negative bands in this region in the film spectra discussed later leads us to believe that the extremum at 167 nm in Figure 1 does represent a resolved CD band. The spectrum of poly(L-proline) II in

water reported by Timasheff et al.⁶ is essentially identical with our spectrum and that of Bovey and Hood⁵ in spite of the difference in solvent. Apparently, as long as the solvent is one in which PPII is the stable form the CD is independent of the solvent.

Our spectrum of a poly(L-proline) II film cast from water (Figure 2) shows that the solution band at 227 nm is shifted to 230 nm, and the solution band at 206 nm is shifted to 214 nm. The crossover, however, remains at 223 nm. The positive band is much stronger in the film spectrum than in solution, relative to the negative band. Mandel and Holzwarth⁷ have reported the CD of a poly(L-proline) film cast from trifluoroethanol from 195 to 240 nm. Their spectrum is more like the solution spectrum than ours is in that they do not observe large band shifts relative to the solution spectrum. In the vacuum ultraviolet region we observe two large negative CD bands at 178 and 146 nm.

Figure 3 shows the results of our time study of the poly(L-proline) I \rightarrow II interconversion in trifluoroethanol. Bovey and Hood⁵ carried out a similar study of the interconversion in trifluoroethanol, reporting solution spectra in the range from 185 to 240 nm. As described above we cast films from the solution after 3 min, 88 min, 179 min, 291 min, and 52 hr. At the earliest time poly(L-proline) I is the predominant form.⁵ Our 3-min spectrum is similar to the CD of PPI reported by earlier works⁵⁻⁷ from 190 to 240 nm. In the vacuum ultraviolet region we find a resolved negative band at 150 nm and a broad negative plateau from 165 to 175 nm. The appearance of a negative band in PPII at 167 nm in trifluoroethanol solution and at 177 nm in a film cast from water leads us to believe that the negative ellipticity in PPI from 165 to 198 nm represents two negative bands which are not well resolved.

As the interconversion proceeds the large positive 216-nm band decreases in intensity. Eventually the ellipticity at this wavelength becomes negative and after interconversion is complete (52-hr spectrum) the large negative band characteristic of PPII appears at 214 nm. The changes in ellipticity at 216 and at 214 nm appear to obey first-order kinetics fairly well yielding an apparent rate constant of $k = 0.0078 \text{ min}^{-1}$ and a half-life for the interconversion of 89 min. Bovey and Hood reported a half-life of 90 min.⁵

The 88-min spectrum therefore represents the midpoint in the interconversion process. At this time the CD spectrum from 190 to 240 nm is very nearly conservative with a pair of oppositely signed bands of approximately equal magnitude. Also at this time a well-resolved negative band appears near 180 nm and as the interconversion goes to completion this band shifts slightly to 178 nm. The large negative band which appears at 150 nm in PPI shifts during the conversion to 146 nm and gradually increases in intensity.

Bensing and Pysh¹⁴ have reported the vacuum ultraviolet absorption spectra of PPI and PPII films prepared under similar conditions. PPI and PPII both have shoulders on the high energy side of the strong absorption band; this shoulder is located near 170 nm, but since it is poorly resolved in both PPI and PPII that location is only approximate. The circular dichroism band we observe here at 178 nm in PPII and about 180 nm in PPI is most likely associated with the same transition. Furthermore this transition is probably the same amide transition that is seen at 160 nm in α helical polypeptides.¹⁴⁻¹⁶ It is interesting to note that this transition has negative ellipticity in α helical polypeptides^{3,4} as well as both PPI and PPII.

The vacuum ultraviolet absorption of PPI and PPII observed by Bensing and Pysh¹⁴ rises sharply below 160 nm, with no resolved bands. However, Young and Pysh³ observed a positive VUCD band at 142 nm in α -helical

poly(L-alanine). That band and the bands we observe here at 146 nm in PPII and 150 nm in PPI most likely have parentage in the same amide transition. It is interesting to note that whereas in the α -helical conformation the rotational strength of the transition is positive, it is negative in both PPI and PPII. This is to be contrasted with the behavior of the transition previously discussed.

The CD spectra of PPI and PPII in the region from 190 to 240 nm have been analyzed with the goal of resolving the observed spectra into their spectral components.^{6,7,17} It is interesting to note that Carver et al.¹⁷ concluded from their analysis of optical rotatory dispersion that there must be a negative CD band in PPI below 185 nm; a conclusion which our data now confirm. Mandel and Holzwarth⁷ constrain their spectral decomposition to be consistent with the absorption spectrum and linear dichroism as well, a technique which reduces the ambiguity of the results.

The low energy CD spectrum of PPI can be assigned on the basis of past calculations⁸⁻¹⁰ and Mandel and Holzwarth's analysis.⁷ The weak negative CD band observed near 238 nm originates in an $n-\pi^*$ amide transition. The large positive band at 214-216 nm and the negative band at 198-199 nm result mainly from exciton interactions among the lowest energy $\pi-\pi^*$ transitions. The small translation per residue in this tight helix (1.85 Å)¹⁸ accounts for the importance of the exciton contribution to CD. Since the positive band is substantially larger than the negative band, however, there must be other, nonexciton contributions as well, since it is known that the exciton contributions, when summed over all components of a transition, must equal zero. A significant part of the positive CD band therefore must come from interactions with higher energy states. Since the CD bands at 150 nm and near 180 nm are both negative, the $\pi-\pi^*$ transition could in principle be coupled to one or both of these transitions. Since the polarization direction of these two transitions is not known, that possibility cannot be explored. The important coupling could also be that with other transitions of even higher energy.

A perturbation treatment of the coupling of the $\pi-\pi^*$ transition with all higher energy transitions through a polarizability approximation has recently been carried out by Ronish and Krimm.¹⁹ The polarizability contribution to $\pi-\pi^*$ CD which they calculate, however, is negative, contrary to the observed net positive dichroism in that region.

The origin of CD in PPII has been discussed several times.^{7-11,19,20} Suffice it to say here that our present data rule out the pure exciton model of Tterlikkis et al.¹¹ If the 228-nm band in PPII is assigned entirely to an $n-\pi^*$ transition, then the 206-nm negative band reflects only the strong nonexciton coupling of the $\pi-\pi^*$ transition with high energy states. If the 228-nm band is assigned to the positive component and the 206-nm band to the negative component of a pair of exciton bands, then the fact that the negative 206-nm band is more intense must also reflect significant additional coupling of the $\pi-\pi^*$ transition with higher energy states via a nonexciton mechanism.

This nonexciton coupling with high energy transitions has in fact recently been recalculated independently by Ronish and Krimm¹⁹ and by Pysh.²⁰ Ronish and Krimm¹⁹ used perturbation theory while Pysh²⁰ used the nonperturbative random phase approximation. Their parameterizations, although similar, were not identical. Both groups came to the conclusion that the nonexciton contribution to $\pi-\pi^*$ CD in PPII is large and significantly alters the exciton-only results. The net calculated CD band shape for the $\pi-\pi^*$ transition is similar to the observed spectrum.

We wish finally to point out that the calculations of Ronish and Krimm¹⁹ and of Pysh²⁰ were carried out for disoriented samples. As we mentioned earlier, the film data re-

ported here gave no evidence of birefringence; but the test for nonbirefringence which we used does not rule out a net orientation perpendicular to the film surface. We think such an orientation is unlikely, but should it be present in our films, a quantitative comparison of the cited calculations with these data would not be appropriate. Nevertheless, insofar as such an orientation is small, a qualitative comparison is still allowed.

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Proton Nuclear Magnetic Resonance Studies of High-Spin Manganese(III) Complexes with Synthetic Porphyrins

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Abstract: The proton NMR spectra of a series of manganese complexes with the synthetic porphyrins, tetraarylporphyrin, octaethylporphyrin, and tetra-*n*-propylporphyrin have been analyzed and assigned. Some discrepancies between the assignments of natural and synthetic porphyrin complexes are noted. The isotropic shifts are shown to be predominantly contact in origin, reflecting extensive porphyrin-to-metal π bonding. This spin transfer mechanism is consistent with the decrease in the extent of spin transfer with increasing π donor properties of the axial halogen. The mechanism of spin transfer appears unaffected upon addition of nitrogenous bases. The bonding in the Mn(III) complexes is compared with that of both high-spin and low-spin ferric porphyrins.

During the past 5 years it has become abundantly clear that NMR spectroscopy in paramagnetic proteins,^{2a} particularly hemoproteins,^{2b} can provide a wealth of information on the electronic, magnetic, and stereochemical properties of the active site. In view of the complexity of the hemoproteins,² parallel advances have been made in analyzing the proton NMR spectra of simple model porphyrin complexes.³⁻⁹ The use of such model compounds has been shown^{2b-9} to facilitate the elucidation and interpretation of the spectral properties of the active site in hemoproteins under conditions which permit some latitude in the variation of the parameters of interest.

Although the bulk of the recent interest in model compounds has focused on the iron porphyrins^{2b-9} due to their occurrence as the prosthetic group in myoglobins, hemoglobins, and cytochromes,^{2a} the demonstrated activity of cobalt-hemoglobin¹⁰ has led to similar interest in the NMR spectra of cobalt(II) porphyrin complexes.^{11,12} Proton NMR studies of the high-spin,^{3,9} HS, and low-spin,⁴⁻⁸ LS, ferric and LS cobalt(II)^{11,12} complexes have permitted the characterization of the metal porphyrin, M-P, π bonding, the magnetic properties of the metal, as well as certain dynamic and thermodynamic properties of axial ligation. Comparison between complexes of natural^{3,4,12} and synthetic^{5-9,11} porphyrin ligands has indicated that the bonding, magnetic, and dynamic properties of interest are very similar and that the complexes of synthetic ligands may serve as useful models for some properties of hemoproteins. Furthermore, the high symmetry and structural variety

available in synthetic porphyrins has been shown to yield less ambiguous assignments as well as interpretations of the coupling constants in terms of M-P π bonding.

Unambiguous evidence for a biological role for manganese porphyrins is lacking at this time. However, the possible role^{13a} of manganese-chlorophyll-type complexes in photosynthesis has been suggested, and recent work indicates the existence of manganese porphyrin in erythrocytes.^{13b} Furthermore, both myoglobin, Mb, and hemoglobin, Hb, can be reconstituted^{13a} with manganese protoporphyrin IX. Although MnMb and MnHb are inactive with respect to reversible oxygenation, hybrid hemoglobins containing both manganese and iron have been demonstrated¹⁴ recently to serve as valuable models for probing the nature of the cooperatively effect.¹⁵ The possibility of detecting¹⁴ hyperfine shifted resonance due to the Mn(III) in such hemoproteins suggests that an elucidation of the origin of the isotropic shifts in model Mn(III) porphyrin complexes may be useful for interpreting the protein spectra. In addition, a comparison^{13a} of the M-P bonding in Mn(III) and Fe(III) complexes may shed some light on the unique role played by iron in these proteins.

Mn(III) porphyrins constitute^{13a} a class of well characterized complexes which have been the center of recent attention¹⁶⁻²⁰ due to unusual optical properties which have been interpreted²¹ as reflecting significant M-P π bonding. The complexes^{13a} are always HS, with $S = 2$, occurring as both five-coordinate,¹⁹ PMn(III)X, and six-coordinate species²⁰ (presumably PMn(III)X(B)) (X = halide or