The Conformational Transition Induced in Poly(4-hydroxy-L-proline) by Calcium Chloride[1]

Wayne L. Mattice[2] and Leo Mandelkern

Department of Chemistry and Institute of Molecular Biophysics,
The Florida State University, Tallahassee, Florida 32306. *Received December 10, 1969*

ABSTRACT: The intrinsic viscosity and circular dichroism of poly(4-hydroxy-L-proline) have been measured at 30° in water and as a function of calcium chloride concentration. The results demonstrate the presence of an ordered structure in pure water which is converted to a random coil in concentrated calcium chloride solutions. The area under the positive Gaussian component of the circular dichroism is the most reliable optical property for following this conformational transition and correlates very well with the intrinsic viscosity measurements. The ordered form of poly(4-hydroxy-L-proline) is more stable to disruption by calcium chloride than poly(L-proline) form II.

Poly(L-proline) can exist in two ordered conformations. Form I contains a *cis*-peptide bond, yielding a right-handed helix,[3] and form II contains a *trans*-peptide bond, leading to the formation of a left-handed helix.[4,5] In certain solvents, including water, the conformation of form II is maintained due to severe steric restriction to rotation about the carbonyl carbon–α-C carbon bond, the one bond per repeating unit capable of some rotational freedom.[6] The hydrodynamic[7–11] and optical[8–14] properties of poly(L-proline) form II are altered by concentrated aqueous solutions of certain neutral salts. It has recently been shown quantitatively that poly(L-proline) attains the properties of a statistical random coil at 2.5–4.0 *M* calcium chloride at 30°. This conformational transition can be monitored by intrinsic viscosity measurements and by appropriate resolution of circular dichroism spectra.[11]

Poly(4-hydroxy-L-proline) can exist in two modifications in the solid state.[15,16] The X-ray pattern of poly(4-hydroxy-L-proline) B exhibits only a few diffuse reflections so that its structure has not been defined. Analysis of the more detailed X-ray patterns obtained from poly(4-hydroxy-L-proline) A reveals[15] that the conformation of the individual molecule is similar to that found in poly(L-proline) form II. The packing of the molecules is altered, however, so that hydrogen bonds between the hydroxyl group on the side chain and a carbonyl oxygen on an adjacent molecule can be formed. The studies reported here were carried out to ascertain whether the introduction of a hydroxyl group into the side chain of poly(L-proline) alters its ordered stability in dilute aqueous solution and if the correlation between the intrinsic viscosity and Gaussian circular dichorism bands, previously reported for poly(L-proline),[11] is maintained. The details of the experimental methods have been described previously.[11]

The intrinsic viscosity of poly(4-hydroxy-L-proline), $\bar{M}_w$ = 9000 at 30°, is shown as a function of calcium chloride concentration in Figure 1. Calcium chloride brings about a reduction in the intrinsic viscosity from 0.29 dl/g in water to 0.08 dl/g in 5.57 *M* calcium chloride. Although there is a very slight decrease in the intrinsic viscosity from 0 to 2 *M* calcium chloride, the major reduction occurs at the higher salt concentrations. For comparative purposes, the intrinsic viscosity of a poly(L-proline) sample of similar intrinsic viscosity ($\bar{M}_n$ = 7400, $\bar{M}_w$ = 9200)[11] is also given in Figure 1. The transition of poly(L-proline) occurs at lower calcium

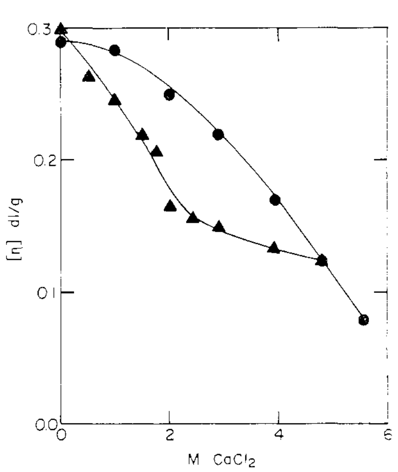

Figure 1. Intrinsic viscosity of poly(4-hydroxy-L-proline) (●) and poly(L-proline) (▲) as a function of calcium chloride concentration at 30°. Both polymers have the same intrinsic viscosity in 4.80 *M* calcium chloride. The data for poly(L-proline) are from ref 11.

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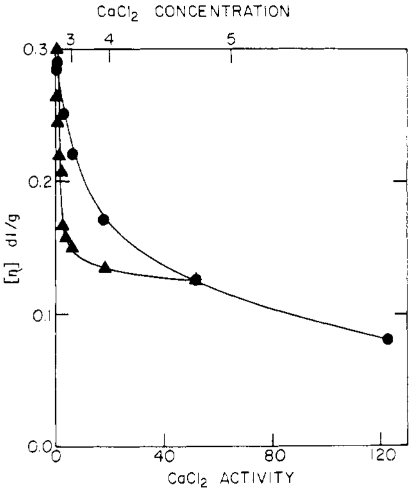

Figure 2. Intrinsic viscosity of poly(4-hydroxy-L-proline) (●) and poly(L-proline) (▲) at 30° as a function of calcium chloride activity. The activity is that of calcium chloride at 25°. The data for poly(L-proline) are from ref 11.

chloride concentrations than does the transition of poly(4-hydroxy-L-proline), and the intrinsic viscosity of the former polymer shows little change above 3 *M* calcium chloride.

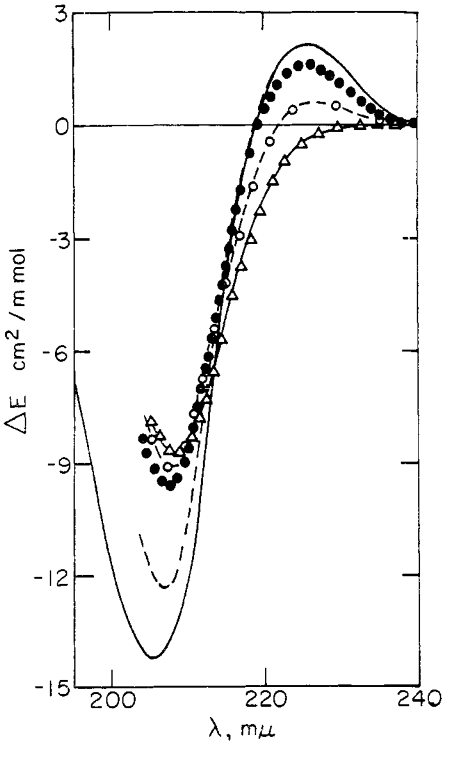

Figure 3. Circular dichrosim of poly(4-hydroxy-L-proline) in water (——), 2.00 *M* (- - -), 3.80 *M* (●●●), 4.80 *M* (-O-O-), and 5.57 *M* (△) calcium chloride.

TABLE I
CIRCULAR DICHROISM OF POLY(4-HYDROXY-L-PROLINE) AND POLY(L-PROLINE) AT 30° IN WATER

Feature[a]	Poly(4-hydroxy-L-proline)	Poly(L-proline)[b]
ΔE_{max}, cm^2/mmol	2.2	0.5
λ_{max}, mμ	225	228
λ_{cross}, mμ	219	224
ΔE_{min}, cm^2/mmol	−14	−13
λ_{min}, mμ	205	206

[a] Subscript max, min, and cross refer to the observed maximum, minimum, and crossover, respectively. [b] Reference 11.

The same data are plotted against the activity of calcium chloride in Figure 2. The activity was calculated from the molal activity coefficient[17] at 25° and tabulated densities of aqueous calcium chloride.[18] The density of 5.57 *M* calcium chloride was estimated by a short extrapolation of the existing data. The change in the intrinsic viscosity of both polymers is most rapid at the lower activities of calcium chloride. However, poly(L-proline) has attained a limiting intrinsic viscosity at activities corresponding to a calcium chloride concentration of about 3 *M*, while the change in the intrinsic viscosity of poly(4-hydroxy-L-proline) is still substantial at activities corresponding to a calcium chloride concentration of 4 *M*. Although a terminal intrinsic viscosity may be approached at the highest calcium chloride activity, the data of Figures 1 and 2 indicate the distinct possibility that the transformation may not be complete.

The circular dichroism of the same poly(4-hydroxy-L-proline) sample in water and aqueous calcium chloride at 30° is shown in Figure 3. The major features of the spectra of poly(4-hydroxy-L-proline) and poly(L-proline) in water at 30° are compared in Table I. The similarity of the two spectra indicates essentially the same ordered chain structure in the two cases. Quantitatively the major difference between the spectra of the two polymers is the apparent increase in intensity and blue shift of the maximum for poly(4-hydroxy-L-proline). The spectra were resolved into two Gaussian curves which reproduce the observed results. The Gaussian parameters obtained for poly(4-hydroxy-L-proline) in water are given in Table II and compared to those of poly(L-proline). In the resolved spectra essentially identical values are obtained for the ΔE at the extremes of the corresponding Guassian curves of the two polymers. However, there is less overlap of the two bands in the spectrum of poly(4-hydroxy-L-proline) because the bands in this polymer are narrower and the band separation may be slightly increased. The areas under the positive Gaussian bands are not significantly different. Therefore the increased size and blue shift of the maximum directly observed in the circular dichroism of poly(4-hydroxy-L-proline) is due to the reduction in the overlap of the positive and negative Gaussian bands.

The addition of calcium chloride causes a decrease in ΔE at the observed maximum and minimum. There is a red shift of the minimum to 209 mμ in 5.57 *M* calcium chloride. Two Gaussian curves of opposite sign were required to fit the spectra obtained at concentrations of calcium chloride of 5.30 *M* or less. A weak positive circular dichroism is still observed in 5.30 *M* calcium chloride (not plotted). Positive circu-

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TABLE II
GAUSSIAN CURVES FITTING THE CIRCULAR DICHROISM OF POLY(4-HYDROXY-L-PROLINE) AND POLY(L-PROLINE) AT 30° IN WATER

Feature[a]	Poly(4-hydroxy-L-proline)	Poly(L-proline)[b]
ΔE_m^+, cm²/mmol	3.35 ± 0.4	3.2 ± 0.6
λ_m^+, mμ	222.8 ± 0.8	221.1 ± 0.9
ω^+, cm⁻¹	1,760 ± 95	2,040 ± 90
Area⁺, cm/mmol	5,250 ± 900	5,950 ± 1350
ΔE_m^-, cm²/mmol	−14.2 ± 0.4	−13.8 ± 0.4
λ_m^-, mμ	205.0 ± 0.2	206.2 ± 0.8
ω^-, cm⁻¹	2,600 ± 95	3,130 ± 90
Area⁻, cm/mmol	32,700 ± 1200	38,300 ± 2200

[a] ΔE_m is the value at the maximum, λ_m is the location of the maximum, ω is the half-width where $\Delta E = \Delta E_m/e$, and area is $0.5(\pi)^{0.5}(\Delta E_m)(\omega)$. Superscript + and − refer to the positive and negative Gaussian curves, respectively. [b] Reference 11. The circular dichroism of poly(L-proline) has been resolved with similar results by J. P. Carver, E. Shechter, and E. R. Blout, *J. Amer. Chem. Soc.*, **88,** 2550 (1966), and S. N. Timasheff, H. Susi, R. Townend, L. Stevens, M. J. Gorbunoff, and T. F. Kumosinski in "Conformation of Biopolymers," Vol. 1, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, p 173.

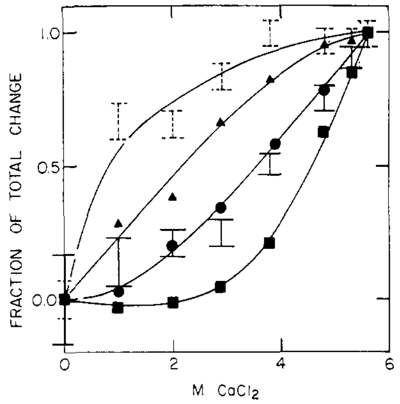

Figure 4. Fractional change between water and 5.57 *M* calcium chloride of the intrinsic viscosity (●), ΔE_{205} (▲), ΔE_{225} (■), and the areas under the resolved negative (dotted bars) and positive (solid bars) Gaussian curves for poly(4-hydroxy-L-proline) at 30°.

lar dichroism is absent in 5.57 *M* calcium chloride, and the observed spectrum in this solvent can be fitted by a single negative Gaussian curve. The absence of the maximum can be taken as evidence[11,13] that the random coil structure has been achieved.

A comparison of the change in the intrinsic viscosity, observed circular dichroism at 205 and 225 mμ, and the areas under the negative and positive Gaussian curves with calcium chloride concentration are presented in Figure 4. It is seen that there is an excellent correlation between the intrinsic viscosity and the area under the positive Gaussian curve for the whole range of salt concentration. However, the changes in the observed circular dichroism at 205 and 225 mμ and the variation in the area under the negative Gaussian curve do not parallel the intrinsic viscosity.

Previously a very good correlation was found, for poly(L-proline), between the changes in the intrinsic viscosity and the area under either of the Gaussian circular dichroism bands.[11] Even though the structural transition as measured by viscosity is different for poly(4-hydroxy-L-proline) and poly(L-proline), the area under the negative Gaussian band in *both* polymers exhibits its sharpest reduction at the lower concentrations of calcium chloride. Only small changes are observed with either polymer above about 3 *M* calcium chloride. The effect of changing the solvent is apparently important in determining the area under the negative Guassian band. Coincidentally, with poly(L-proline) this effect occurs at the same salt concentration as the conformational transition measured by the intrinsic viscosity. In general, therefore, correlation of the structural changes exists only between the intrinsic viscosity and the area under the resolved positive circular dichroism band.

The observation that the ordered structure assumed by poly(4-hydroxy-L-proline) in water is more stable to disruption by calcium chloride than is form II of poly(L-proline) must be due to either a greater stability of the isolated poly(4-hydroxy-L-proline) helix or the result of intermolecular interaction arising from association of the molecules in solution. There does not appear to be any theoretical basis to expect an increased stability of the isolated chain. The possible formation of dimers[19] and trimers[20] has been previously suggested.

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