Dynamics of the Helix–Coil Transition in Poly-L-ornithine[1]

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Abstract: Ultrasonic attenuation measurements have been carried out in solutions of poly-L-ornithine in water (0.2 *M* NaCl)–methanol (85:15) at 25° over the frequency range 10–165 MHz and the pH range 10–11.85. A single relaxation process is observed, and the associated relaxation time and maximum chemical absorption go through a maximum at a pH where circular dichroism measurements indicate a helix–coil transition is occurring. The maximum value of the relaxation time is 1.7×10^{-8} sec. The observed relaxation process appears to be directly associated with the kinetics of the helix–coil transition, and the data are in quantitative agreement with current theories of the dynamics of this transition. The rate constant for formation of a helical segment at the end of an already helical section is estimated to be 7.4×10^{10} sec^{-1}, and the volume change associated with this process is approximately ±0.6 cc/mol of monomer.

The reversible transition between α-helical and randomly coiled conformations of polypeptides in solution has been intensively investigated in recent years (*cf.* ref 2 for comprehensive reviews of this subject). These transitions serve as useful models for cooperative structural changes found in more complex biological systems. Typically the transition occurs over a very small range in some property of the system such as pH or temperature. The equilibrium properties of the transition have been studied by a variety of experimental techniques and many theoretical treatments of the transition have also been developed.[2]

In contrast to the wealth of information on the helix-coil equilibrium, very little is known about the dynamics of the transition process. Bounds for the average relaxation time, τ^*, near the midpoint of the transition for poly-L-glutamic acid in water and water–dioxane mixtures have been estimated by the temperature jump[3] and ultrasonic attenuation techniques[4] as $10^{-5} > \tau^* > 5 \times 10^{-8}$ sec. Parker, *et al.*,[5] have investigated the ultrasonic attenuation of aqueous solutions of poly-L-lysine as a function of pH and ionic strength. The observed relaxation time of 3–6 $\times 10^{-8}$ sec was suggested to be associated with the helix–coil transition. Because of the restricted pH range investigated, however, the nature of the relaxation process could not be established with certitude. Recent dielectric relaxation measurements of poly-γ-benzyl-L-glutamate in dichloracetic acid–ethylene dichloride mixtures have indicated the average relaxation time at the midpoint of the transition is about 5×10^{-7} sec.[6] Although few experimental data concerned with the dynamics of the helix–coil transition are available, several theoretical treatments of the kinetics of this process have been published[7,8] and experimental verification of these theories would be of interest.

In the work presented here, the ultrasonic attenuation of poly-L-ornithine solutions was measured over the frequency range 10–165 MHz as a function of pH. The solvent used was water (0.2 *M* NaCl)–methanol (85:15, v/v); the inclusion of methanol in the solvent increases the solubility of the polypeptide and substantially raises the amount of helical structure formed. Poly-L-ornithine is known to undergo conversion from the coiled to helical conformation on raising the pH.[9,10] In aqueous solution the estimated maximum amount of helical structure is about 25% but increases to about 90% in 90% methanol.[10] This particular polypeptide was selected for investigation because of the low thermodynamic stability of its α-helical conformation; it was hoped that this low stability might be reflected in an increase in the rates of the relaxation processes associated with the helix–coil transition relative to poly-L-glutamic acid since the rates for the latter compound are too slow for measurement with the ultrasonic equipment at our disposal.

Experimental Section

Poly-L-ornithine (Pilot Chemicals) was obtained as the hydrobromide in two lots, 0–19 (molecular weight ~94,000) and 0–21 (molecular weight ~74,000). Two series of experiments were performed: in the first untreated 0–19 was used, while in the second series 0–19 with less than 20% 0–21 by weight was used after dialysis in the presence of chloride and lyopholization. Fresh bottles of analytical reagent grade methanol (Mallinckrodt) and sodium chloride (Baker and Adamson) were used. Saturated sodium hydroxide and concentrated hydrochloric acid were used to adjust the pH in order to maintain an approximately constant polymer concentration throughout the experiments.

Circular dichroism measurements were made on a Jasco ORD-CD-UV5 over the wavelength region 205–250 mμ. The technique for measuring the ultrasonic attenuation has been previously described;[4,11] the frequency range covered was 10–165 MHz. A Radiometer pH meter with a GK 2021C electrode was used to

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[1] This work was supported by a grant from the National Institutes of Health (GM13292).

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measure the apparent pH. Sodium ion corrections were applied at pH values greater than 10.

All experiments were performed at 25°. The solvent was water (0.2 *M* NaCl)-methanol (85:15, v/v). The polymer concentration used was approximately 0.04% for the optical measurements and approximately 2% for the ultrasonic attenuation measurements. Actual concentrations were determined by micro-Kjeldahl analysis. The circular dichroism and ultrasonic attenuation were measured over the pH range covered in two separate series of experiments, one using the untreated hydrobromide polypeptide, the other the dialyzed and lyopholized polypeptide. An average of pH values recorded immediately before and after measurement of the circular dichroism or ultrasonic attenuation frequency spectrum was used. Circular dichroism recordings were complete within 10 min of pH adjustment. However, an ultrasonic attenuation frequency spectrum takes a period of a few hours to measure. The ultrasonic cell was sealed to the air as much as possible and the spectrum recorded as rapidly as possible. Even at high pH values, the solution pH never fell by more than 0.06 unit during a measurement. The pH was not progressively increased or decreased, but was varied randomly; generally a low pH value was followed by a high pH value and then another relatively low value. This procedure reduced the possibility of any time-dependent phenomena influencing the interpretation of the final results. The pH was not raised above pH 11.85 as significant hydrolysis would probably occur over the time period necessary for obtaining an ultrasonic relaxation spectrum, and the solubility of the polypeptide decreases with increasing pH.

Results

The circular dichroism spectra were essentially the same as previously published[10] and will not be presented here. The results, however, will be used later to calculate the fraction of the polymer in a helical conformation.

The water (0.2 *M* NaCl)-methanol (85:15) solvent in the absence of the polypeptide was found to possess a constant value of α/f^2 of 21.9 (±0.5) $\times 10^{-17}$ cm^{-1} sec^2 throughout the frequency range 10–165 MHz. Here α is the pressure amplitude attenuation coefficient and f is the frequency of the ultrasonic radiation. For a mixed solvent such as this, the measured pH cannot be directly interpreted in terms of the hydrogen ion concentration; however this is of no consequence in this work since pH is only used as a parameter to adjust the ratio of helical to randomly coiled configurations. The frequency spectrum of a 2% poly-L-ornithine solution was studied in the apparent pH range 10.04–11.85. At all pH values a relaxation process was observed which was consistent with the equation for a single relaxation process, namely[12]

$$\alpha/f^2 = \frac{A\tau}{1 + (\omega\tau)^2} + B \qquad (1)$$

where τ is the relaxation time, ω $(= 2\pi f)$ is the radial frequency and A and B are constants. Equation 1 can be written in terms of the chemical absorption per wavelength, μ_{ch}, as[13]

$$\mu_{\text{ch}} = 2vf(\alpha/f^2 - B) = \frac{2\mu_{\text{m}}\omega\tau}{1 + (\omega\tau)^2} + B \qquad (2)$$

where v is the ultrasonic velocity and μ_{m} $(= Av/2\pi)$ is the maximum chemical absorption per wavelength. Some typical data are shown in Figure 1 where the variation of μ_{ch} with frequency is recorded at pH 10.43 and 11.38

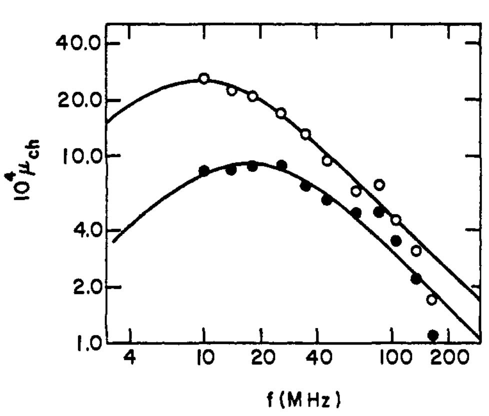

Figure 1. Chemical absorption per wavelength, μ_{ch}, *vs.* frequency, *f*, for 2% poly-L-ornithine in water (0.2 *M* NaCl)-methanol (85:15, v/v) at 25°: pH 11.38, ○; pH 10.43, ●. The curves are calculated according to eq 2 with $\mu_{\text{m}} = 2.5 \times 10^{-3}$ and $\tau = 1.7 \times 10^{-8}$ sec at pH 11.38 and $\mu_{\text{m}} = 0.92 \times 10^{-3}$ and $\tau = 9.3 \times 10^{-9}$ sec at pH 10.43.

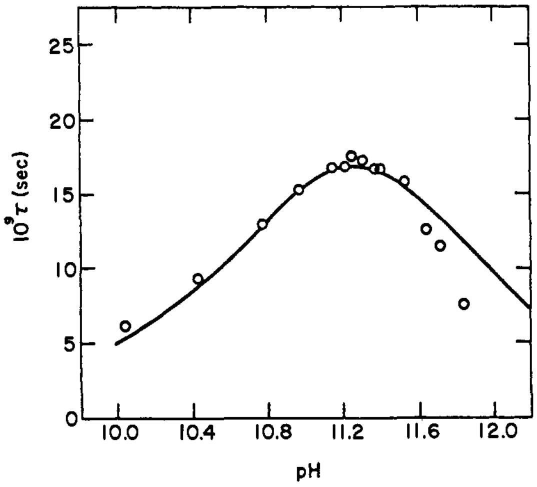

Figure 2. Relaxation time, τ, *vs.* pH for 2% poly-L-ornithine in water (0.2 *M* NaCl)-methanol (85:15) at 25°. The curve is calculated as described in the text.

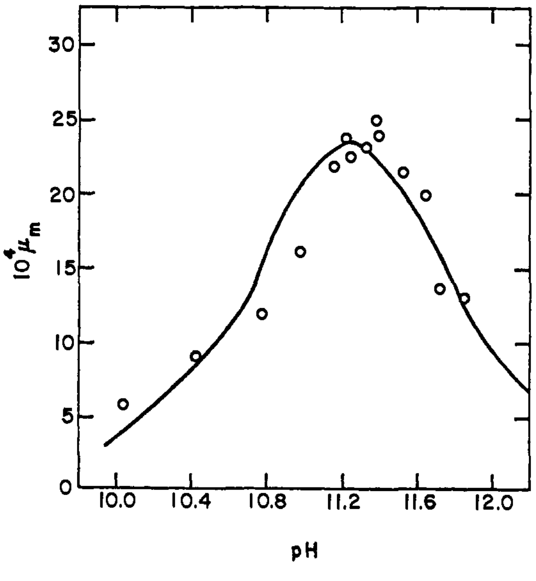

Figure 3. Maximum chemical absorption per wavelength, μ_{m}, *vs.* pH for 2% poly-L-ornithine in water (0.2 *M* NaCl)-methanol (85:15) at 25°. The curve is calculated as described in the text.

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together with the theoretical curves calculated according to eq 2. The ultrasonic parameters μ_m and τ were obtained by the method of Piercy and Subrahmanyam,[14] and the estimated uncertainty in these parameters is 10–15%. Figures 2 and 3 show the variation of the relaxation time and the maximum chemical absorption per wavelength with the measured pH. Data from both series of experiments at a monomer concentration of 0.114 M are included in the figures. The curves are theoretical and are calculated as outlined below. The limiting value of α/f^2 at high frequencies, B, was between 24–26 × 10^{-17} cm^{-1} sec^2 at all pH values and the sound velocity was 1.64 (±0.02) × 10^5 cm/sec.

Theory

A brief outline of the relevant equilibrium and kinetic theory of the helix–coil transition is presented here as a basis for the interpretation of the experimental results. The Zimm–Bragg model[15] was chosen as the most convenient theory of the equilibrium process for our purposes. This theory contains two parameters, the equilibrium constant for the addition of a helical segment to another helical unit, s, and the equilibrium constant for the formation of a helical unit to a randomly coiled unit, σs. The parameter σ is thus a measure of the degree of difficulty of nucleation, and for a cooperative process $\sigma \ll 1$. For long chain polymers, the fraction of polymeric units in the helical configuration, θ, is given by

$$\theta = \frac{1}{2}\left[1 + \frac{s-1}{\{(1-s)^2 + 4\sigma s\}^{1/2}}\right] \quad (3)$$

The midpoint of the transition ($\theta = 0.5$) is found when $s = 1$.

A kinetic theory utilizing the Zimm–Bragg model has been developed by Schwarz.[7] In his treatment, the equilibrium constant s is set equal to k_F/k_B where k_F and k_B are the rates of formation and disintegration of a helical unit at the end of an already helical segment. A spectrum of relaxation times is to be expected because of the large number of possible states of the polymer chain involved in the transition. However, the mean relaxation time, τ^*, was shown to be

$$\tau^* = \{k_F[(s-1)^2 + 4\sigma]\}^{-1} \quad (4)$$

The mean relaxation time reaches its maximum value of $1/4\sigma k_F$ when $s = 1$.

The maximum amplitude of the chemical absorption, μ_m, is a function of thermodynamic variables only and for a single reaction under the experimental conditions employed is given to an adequate approximation by[13]

$$\mu_m = \frac{\pi \rho v^2 c_0 \Gamma}{1000RT}[\Delta V - \beta \Delta H/\rho c_P]^2 \quad (5)$$

where ρ is the solution density, R is the gas constant, T is the temperature, c_0 is the molar concentration of monomer, ΔV and ΔH are the volume and enthalpy changes associated with the relaxation process, β is the coefficient of thermal expansion of the solvent, and c_P is the constant pressure specific heat of the solvent. If the reaction characterized by the mean relaxation time is associated with the equilibrium constant, s, then

$$\Gamma = \frac{\partial \theta}{\partial \ln s} = \frac{s\sigma(s+1)}{[(1-s)^2 + 4\sigma s]^{3/2}} \quad (6)$$

where eq 3 has been used to evaluate the derivative. This equation predicts that for a given polymer concentration, μ_m will go through a fairly sharp maximum at $s = 1$.

Thus the theoretical treatment predicts that to a first approximation all of the equilibrium and kinetic properties of the helix–coil transition for long-chain polypeptides can be defined by the parameters s, σ, and k_F. Moreover both τ^* and μ_m should reach a maximum at the midpoint of the transition.

Discussion

Poly-L-ornithine is clearly involved in the observed chemical relaxation since α/f^2 for the solvent alone was a constant throughout the frequency range employed. Only three mechanisms are likely causes for the observed relaxation: protolytic reactions, solvolytic reactions, and the helix–coil transition. For any reasonable solvolytic and protolytic processes, τ and μ_m would be expected to be monotonic functions of pH. Figures 2 and 3 clearly indicate that both of these parameters reach a maximum at a pH of approximately 11.2–11.3. The variation in μ_m and τ is large and well outside experimental error. Therefore, in accord with the theoretical predictions discussed above, the relaxation process observed is probably due to the helix–coil transition.

To assign the relaxation process to the helix–coil transition more definitely, the experimental data can be quantitatively analyzed in terms of the theories previously outlined. For this analysis the variation of the fraction of polypeptide in the helical configuration, θ, with pH must be known. If the midpoint of the transition is assumed to occur at the pH at which τ and μ_m attain their maximum values, and if the molar ellipticity at 222.5 mμ is assumed to be a linear function of θ, then θ can be computed from the equation

$$\theta = \frac{[\theta] - [\theta]_0}{2\{[\theta]_{50} - [\theta]_0\}} \quad (7)$$

Here $[\theta]$ is the observed molar ellipticity at any pH, $[\theta]_{50}$ is the molar ellipticity at the pH equivalent to the midpoint of the transition and $[\theta]_0$ is the molar ellipticity of the randomly coiled configuration (*i.e.*, at neutral pH). The θ *vs.* pH curve obtained in this manner is shown in Figure 4. The experimental values of θ reach a maximum at about $\theta = 0.6$, which is in qualitative agreement with the behavior observed by Chaudhuri and Yang.[10] A curve for a hypothetical complete transition is included in Figure 4 (solid line). This extrapolated curve is useful in fitting the ultrasonic data to theoretical curves and was obtained by assuming symmetry about the midpoint of the transition. Equation 7 was used to determine θ rather than the usual empirical equations[16] since the estimation of the molar ellipticity of a completely helical structure

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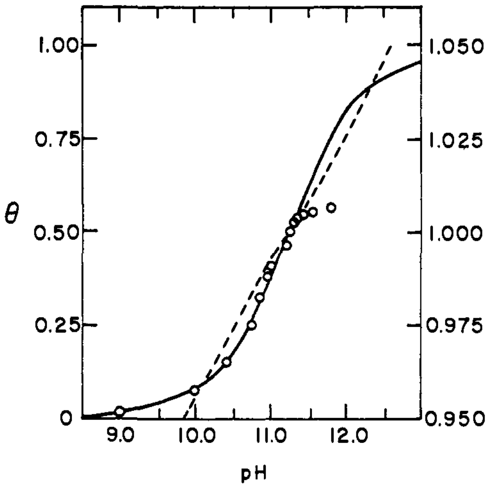

Figure 4. Fractional amount of helix, θ, *vs.* pH for 0.04% poly-L-ornithine in water (0.2 M NaCl)–methanol (85:15) at 25°. The points were obtained from eq 7 and the circular dichroism data, and the solid line is calculated assuming a curve symmetrical about $\theta = 0.5$. The dashed line shows s as a function of pH. Values of s were calculated from eq 3 with $\sigma = 2 \times 10^{-4}$ and values of θ obtained from the solid line curve.

for polypeptides in nonaqueous solvents is subject to considerable uncertainty.[17,18]

According to the theory of Zimm and Bragg[15] the equilibrium properties of the helix–coil transition are characterized by two independent parameters, σ and s (see eq 3), but unfortunately a unique set of parameters cannot be derived for poly-L-ornithine from the existing data. If the value of σ is known, values of s at various pH values can be derived from eq 3 and the curve of θ *vs.* pH given in Figure 4. The value of σ found for poly-γ-benzyl-L-glutamate was 2×10^{-4}.[19] Since the value of σ is mainly dependent on geometrical factors, and is generally regarded as relatively invariant for different polypeptide–solvent systems,[15,20] a value of 2×10^{-4} was also chosen for poly-L-ornithine in this study. Although the assumption of this value cannot be strictly justified, it is probably the right order of magnitude. The variation of s with pH derived from this value of σ and the variation of θ with pH is included in Figure 4 (dashed line).

The dependence of τ and μ_m on pH is determined solely by the constant σ and the variable s (see eq 4–6). With these parameters chosen as above and maximum values of τ and μ_m taken from the experimental data, theoretical curves for the variations of τ and μ_m with pH were calculated according to eq 4–6 and are shown in Figures 2 and 3. Slight adjustments were made in the value chosen for the pH at the midpoint of the transition and the maximum values of τ and μ_m to achieve a good fit between the experimental data and theoretical curves. A good fit was achieved with a pH at the midpoint of 11.25 and maximum values of τ and μ_m of 1.7×10^{-8} sec and 1.35×10^{-3}, respectively. From the maximum value of the relaxation time, k_F is found to be 7.4×10^{10} sec^{-1} (eq 4). The quantity $\pm[\Delta V - \beta\Delta H/\rho c_P]$ can be determined from the maximum value of μ_m (eq 5) and is ±0.6 cc/mol of monomer. If $\Delta H < 1$ kcal/mol, as is very likely for the propagation of the helix in this solvent, then this quantity is, to a good approximation, equal to ΔV.

The agreement between the experimental data and theoretical curves are remarkably good when the approximations in the theory and the experimental error involved are considered. The ultrasonic data are fit to a single relaxation curve, although a spectrum of relaxation times is expected. The distribution of relaxation times is apparently so narrow that within experimental error the distribution can be approximated as a single relaxation time. In correlating these results with the theory, the experimentally measured relaxation time is equated to the mean relaxation time of the theory. In interpreting the amplitude of the ultrasonic relaxation, a single equilibrium constant, s, is assumed to describe the mechanism. This is equivalent to neglecting nucleation or end effects; these effects apparently never dominate the kinetic behavior of the helix–coil transition.[8]

Unfortunately the agreement between theory and experiment is not very dependent on the particular value chosen for σ. Calculations were carried out with other values of σ, and as long as $\sigma \ll 1$, the fit between theory and experiment can be made quite good by appropriate adjustment of s according to eq 3. The values of k_F and ΔV derived, however, are dependent on the value chosen for σ. The rate constant for helix propagation, 7.4×10^{10} sec^{-1}, is similar to that estimated for the formation of a hydrogen-bonded dimer after the monomers have diffused together ($\sim 10^{11}$–10^{12} sec^{-1}).[21] Most studies of the dynamics of hydrogen bonding have been in inert solvents, although the existing data suggest that this rate constant is not markedly solvent dependent. The value of k_F measures the rate of formation of a helical turn in the chain and reflects interactions other than hydrogen bonding; for example hydrophobic interactions and solvation may be of importance. Therefore this value of k_F may vary substantially for different polypeptides. A maximum value for the relaxation time of poly-γ-benzyl-L-glutamate in dichloracetic acid–ethylene dichloride has been estimated as 5×10^{-7} sec,[6] which leads to a value for k_F of 2.5×10^{9} sec^{-1} (with $\sigma = 2 \times 10^{-4}$). This lower value of k_F may reflect a solvation effect, the greater stability of the polybenzylglutamate helix relative to that of poly-L-ornithine, or differences in σ for the polypeptide–solvent systems. The calculated value of the volume change associated with the relaxation process, ±0.6 cc/mol of monomer (assuming $\Delta H < 1$ kcal/mol) is in excellent agreement with the volume change associated with the helix–coil transition of poly-L-glutamic acid, 0.5–1 cc/mol of monomer.[22] (The sign of the volume change cannot be determined from ultrasonic data since the volume change is obtained as a square root.) Thus the value of σ chosen in accord with the value found experimentally for another polypeptide system leads to values of k_F and ΔV consistent with other known data.

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Nevertheless, until an independent determination of one of these three parameters has been made for this particular system, these values must be regarded as quite tentative.

The ultrasonic data indicate that the helical content of the chain is increasing above pH 11.4, although some divergence from the theoretical curves is observed. However, the circular dichroism data suggest that the amount of helix tends to reach a limiting value at this pH. Hydrolysis of the peptide chain above pH 11.85 does not allow accurate ultrasonic data to be obtained above this pH to see if a helical fraction greater than $\sim$0.7 is predicted by the ultrasonic data. Also if θ is obtained from the molar ellipticity by the use of the empirical equations used by Yang,[10,16] the predicted maximum in τ and μ_m occurs at pH 10.95 rather than 11.25 as inferred from the ultrasonic data. However, the uncertainty involved in using such empirical equations in nonaqueous solvents has already been pointed out. No ready explanation of these discrepancies exists, although the two techniques certainly must measure different properties of the polymer.

In the region of pH 9 a small chemical relaxation still exists. The amplitude of this relaxation is far smaller than the amplitudes observed nearer the transition midpoint, but is larger than predicted by an extrapolated value of θ and eq 5 and 6. This may indicate the existence of a small amount of helical content even at high degrees of protonation of the polypeptide side chains, or may be due to the fact that some of the theoretical equations used are not valid far from the midpoint of the transition.

In summary, ultrasonic attenuation measurements of poly-L-ornithine appear to measure directly the dynamics of the helix–coil transition; moreover the pH dependence of the relaxation time and the amplitude of the relaxation process are in quantitative agreement with the theoretical predictions. The rates associated with this polypeptide helix–coil transition can be regarded as approximate upper bounds for the rates of similar, but usually more complex, cooperative processes in proteins.

Conformational Aspects of Polypeptide Structure. XXX. Rotatory Properties of Cyclic and Bicyclic Amides. Restricted and Rigid Model Compounds for Peptide Chromophores[1]

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Abstract: The optical properties of monocyclic and bicyclic amides have been examined by optical rotatory dispersion (ORD) and circular dichroism (CD) techniques. The signs of the rotational strength of the n–π^* transitions of L-α-aminocaprolactam and L-pyrrolid-2-one-5-carboxylic acid are interpreted in terms of both quadrant and octant rules. The effect of charged side-chain groups on the amide chromophores is also discussed. In addition to n–π^* and π–π^* Cotton effects, a third band in the spectra of bicyclic lactams has been observed. Infrared and circular dichroism dilution studies demonstrate that this Cotton effect arises from association of the lactams to form dimers. We have also been able to separate solvent and conformational effects on the ORD and CD spectra for the completely rigid bicyclic amides since alterations in conformation for these compounds can not occur.

Current attention of far-ultraviolet optical rotatory dispersion (ORD) and circular dichroism (CD) for polypeptides and proteins arises from substantial improvements in instrumentation.[4] Recent review articles include discussions on the nature of electronic transitions in the peptide (amide) chromophore.[5–10] We and others[5,6,9,10] believe that the clearest approach to assignment of the amide transitions comes from using low molecular weight optically active compounds. A limited number of such studies has appeared.[9–18]

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(2) Postdoctoral fellow from the Department of Organic Chemistry, University of Padua.

(3) Submitted by Joseph Falcetta to the faculty of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the Ph.D. in Chemistry.

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