that this band would be more sensitive to the physical properties of the R group and less sensitive to chain length. The low magnitude of the long-wavelength CD extremum of poly-L-alanine may be a reflection of this. Recent work on pyrrolidones demonstrates a strong dependence of magnetic transitions on changing properties of perturbing vicinal groups,[6] and it is proposed that data may be analyzed in terms of partial molar rotatory powers of a vicinal group as it applies to the total rotational strength of a magnetic transition. Should this approach be tractable, poly-L-alanine would serve as the reference polypeptide.[36]

The effect of solvent may be to induce a change in the polypeptide backbone conformation; it may be to alter the conformation and charge of the side chain; it may be to change the positions and intensities of the transitions; and it may affect the interactions between transitions and groups by a medium dielectric effect. The random terpolymer ala-glu-lys (1:1:1) is soluble in water over a wide range of pH, and it is soluble in trifluoroethanol. Figure 4 contains the CD data of the terpolymer in TFE–H_2O (90:10 v/v) and in water at pH 1.3. It is seen that there are substantial changes in the actual magnitudes as well as the relative magnitudes of the two negative bands. In TFE the parallel band is more intense whereas the n–π^* band is more intense in H_2O at pH 1.3. In the latter case the curve looks very much like that of myoglobin and poly-L-glutamic acid. At low pH the R groups have a net positive charge while in TFE they are uncharged. The differences in the long-wavelength extremum are in accord with vicinal effects for n–π^* transitions of polypeptides and proteins[37] in that a positively charged R group would be expected to increase the negative rotation of the n–π^* band. The differences observed in the parallel band are more difficult to discuss and may be the result of changes in coordinates of the polypeptide, *e.g.*, α helix to 3_{10} helix, or may more reasonably be the result of other effects of solvent. The several polypeptides which we have examined in TFE, *i.e.*, poly-L-alanine, poly-γ-methyl-L-glutamate, gramicidin S, exhibit the enhanced parallel band.

Acknowledgments. Sincere thanks are due to Miss Leslie Zimmerman for typing the manuscript and for drawing the figures. The technical assistance of Mr. A. Ruiter is also acknowledged.

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(36) NOTE ADDED IN PROOF. Poly-L-alanine has been used as the model polypeptide and the approach appears tractable. Applying the concept of partial molar rotatory powers the rotational strength of the n–π^* transition has been calculated for poly-L-alanine in the β conformation and chain lengths have been predicted (D. W. Urry, *Proc. Natl. Acad. Sci. U. S.*, in press). Also the rotational strength of the n–π^* transition of poly-L-alanine in the α helix has been calculated and predictions on chain-length effects and values for the 3_{10} helix will be forthcoming (D. W. Urry, in preparation).

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Ultraviolet Rotatory Properties of Polypeptides in Solution. II. Poly-L-serine

F. Quadrifoglio[1a] and D. W. Urry[1b]

Contribution from the Institute for Biomedical Research, Education and Research Foundation, American Medical Association, Chicago, Illinois 60610.

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Abstract: Circular dichroism and absorption data are presented for low molecular weight poly-L-serine. Ellipticity extrema are observed in 80% trifluoroethanol at 222 and 197 mμ with molar ellipticities of -0.99×10^4 and 5.2×10^4, respectively. The data are resolved into a set of component Gaussian functions. Resolved circular dichroism curves are centered at 222 and 197 mμ with rotational strengths of -5.6×10^{-40} and 32×10^{-40}, respectively. Resolved absorption curves were obtained at 214, 197, and 184 mμ with dipole strengths of 0.9×10^{-36}, 2.85×10^{-36}, and 6.8×10^{-36}, respectively. The major absorption was in the 184-mμ band and appeared to have little or no corresponding rotational strength. The long-wavelength CD band, likely an n–π^* transition, appeared to be at longer wavelengths and narrower than the corresponding absorption curve, in accordance with predictions of Moffitt and Moscowitz for magnetically allowed transitions. The point is made that β-type CD patterns are variable as to magnitude and position of the extrema when comparing different polypeptides as well as when studying solvent and temperature effects on the same polypeptide. Comparing the molar ellipticity of the long-wavelength CD band of poly-L-serine to that of L-5-hydroxymethylpyrrolid-2-one and considering the structures involved lead to the conclusion that the hydroxymethyl side chains provide significant perturbations contributing to the rotational strength of the long-wavelength transition. The poly-L-serine data, due to the absence of side-chain absorptions in the wavelength range studied, are presented as a set of reference properties characterizing a β conformation.

X-Ray diffraction studies have demonstrated the occurrence of two types of ordered protein structures, helical and pleated sheet. An α helix has been observed in proteins[2] and polypeptides,[3,4] and short

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(1) (a) Postdoctoral Fellow, Oct 1966–Sept 1967, supported by the Education and Research Foundation of the American Medical Association. (b) Author to whom requests for reprints should be addressed.

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segments of threefold helix are reported to occur in lysozyme.[5] The absorption and optical rotation properties of helical structures have been well characterized[6–8] though one cannot be entirely sure whether poly-L-alanine in trifluoroethanol is in the α or threefold helical conformation. The presence of antiparallel pleated sheet in lysozyme[5] and of β structure in β-keratin[9] and silk fibroin[10] has also been shown by X-ray analysis. Of most direct concern to this communication are studies on films and powders of poly-L-serine and its acetyl derivative. Films of poly-O-acetyl-L-serine have been reported on the basis of X-ray as well as infrared studies to be in the cross-β conformation,[11] and infrared data on poly-L-serine (PLS) in the solid state are interpreted to indicate the β conformation.[11,12]

Characterization of the optical rotatory properties of the various β structures is inherently a difficult task because of the limited solubility of these structures in solvents which do not disrupt their conformational order. Davidson, *et al.*,[13] Sarkar and Doty,[14] and Townend, *et al.*,[15] however, have determined the optical rotatory dispersion (ORD) and circular dichroism (CD) spectra of aqueous solutions of poly-L-lysine in the β conformation; Iizuka and Yang have published data on silk fibroin.[16] The CD spectra show a negative band at 217–218 mμ and a positive band at 195–198 mμ. The two bands in poly-L-lysine are approximately the same magnitude with molar ellipticities of 2×10^4. It is reasonable to consider that the long-wavelength band may be due to the n–π^* transition, although Rosenheck and Sommer[17] believe it unlikely that the entire band is due to the n–π^* transition but rather that it may contain an exciton contribution from the NV_1 transition of the monomer. There is agreement that the 195–198-mμ band arises from excitation resonance interactions of the NV_1 transition,[17,18] yet theoretical treatments are in disagreement as to whether the CD and absorption patterns of poly-L-lysine are those of the parallel or antiparallel β structure.[17,18] Similarly interpretations of ultracentrifugation[14] and rate data[19] vary as to whether observed spectra correspond to that of the parallel or antiparallel conformations.

In the first paper of this series, ultraviolet absorption and circular dichroism spectra of helical poly-L-alanine were presented as a characterization of the simplest optically active polypeptide in helical conformation.[8] In the present paper we wish to report data obtained on a low molecular weight sample of poly-L-serine dissolved in water and in water–organic solvent mixtures. On the basis of circular dichroism and infrared studies, it would appear that low molecular weight poly-L-serine is in the β conformation in solution as has been previously noted to be the case in the solid state.[11,12] The advantage of a careful characterization of the optical properties of poly-L-serine lies in the fact that there are no electronic transitions in the side chain which would complicate analysis of the absorption and circular dichroism data to 190 mμ. Specifically, resolved bands are observed at 222 and 197 mμ with molar ellipticities of -9.9×10^3 and 52×10^3, respectively, and resolved absorption bands are seen at 214 and 197 mμ with molar extinction coefficients of 0.6×10^3 and 3.5×10^3, respectively.

Experimental Section

All the solvents were spectrograde Matheson Coleman and Bell products. Lithium chloride was obtained from J. T. Baker. CD measurements were carried out on a Cary Model 60 spectropolarimeter equipped with a CD attachment, Model 6001, built by Cary Instruments. A spectral band width of 15 Å was used. Unless otherwise stated, the temperature was 25°. Scan speeds, pen periods, and time constants were chosen to maintain sufficient response time and to allow adequate signal-to-noise ratios. The CD unit was calibrated by using the Cary Model 1401 circular dichroism attachment for the Model 14. The standard used was an aqueous solution of *d*-10-camphorsulfonic acid (J. T. Baker, Lot No. 9-361) with an $\epsilon_L - \epsilon_R$ of 2.20 ± 0.05 at 290 mμ. Cell path lengths were calibrated with solutions of chromate in 0.05 *N* KOH. Sample temperatures were maintained with the Haake KT-62 Kryothermat and were monitored with a YSI Model 42SC telethermometer while spectra were being run. Absorption curves were determined on a Cary Model 16 spectrophotometer. The stray light of this instrument is less than 0.1% over the operating range (800–170 mμ). Nitrogen was flushed through the Cary Model 16 and Model 60 throughout the measurements. Ir spectra were recorded with a Beckman IR-11 containing the IR-12 interchange. KRS-5 windows were used in a variable path length cell. Curve resolution was performed with a Du Pont 310 curve resolver.

Poly-L-serine was obtained from Yeda (Rehovoth, Israel, Lot SE-22). Its average degree of polymerization was given at 20. At room temperature the polymer is not soluble in water but dissolves when heated to 70°. On cooling no precipitation is observed even after several months. The concentrations of the solutions were determined by nitrogen content using a Coleman 29 nitrogen analyzer. Bohak and Katchalski[12] have shown that optically pure, high molecular weight poly-L-serine is insoluble in water, while the presence of a few D residues in the polymer give rise to a water-soluble product; for example, high molecular weight poly-L-serine is solubilized in water at room temperature by the presence of 5% D residues.[12] As the sample used in this study is of low molecular weight and is not solubilized in water at room temperature, it may be assumed that racemization, if present, is considerably less than 5%.

Results and Discussion

The CD spectrum of poly-L-serine immediately after dissolution in water at 70° shows a negative band at 222 mμ and a positive band at 197 mμ. CD measurements obtained at room temperature and repeated at fixed time intervals after dissolution showed the amplitude of the two peaks to increase until a constant value was reached. The CD curve registered at equilibrium is shown in Figure 1. In the same figure the CD spectrum of poly-L-serine dissolved in 8 *M* LiCl is seen. As high concentrations of lithium salts destabilize the ordered structures of polypeptides and proteins,[20,21] the CD

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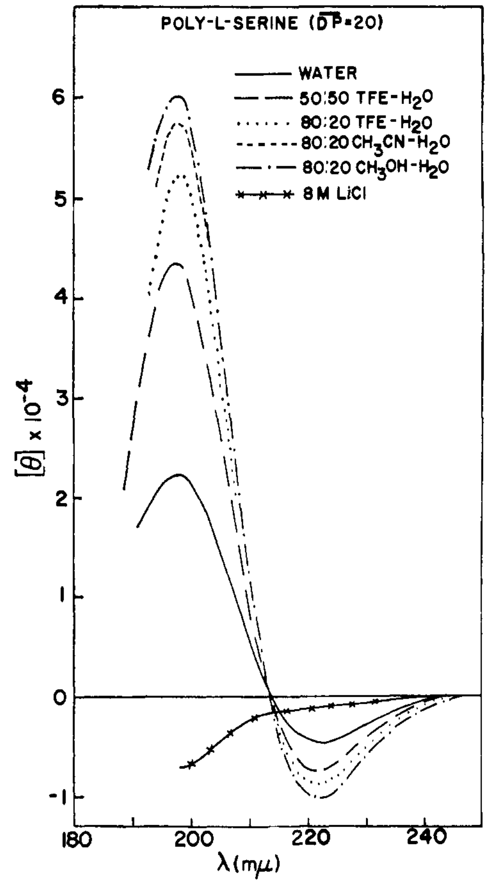

Figure 1. Circular dichroism in the 250–190-mμ range of poly-L-serine in water and several water–organic solvent mixtures. Also included is a curve for PLS in 9 M LiCl.

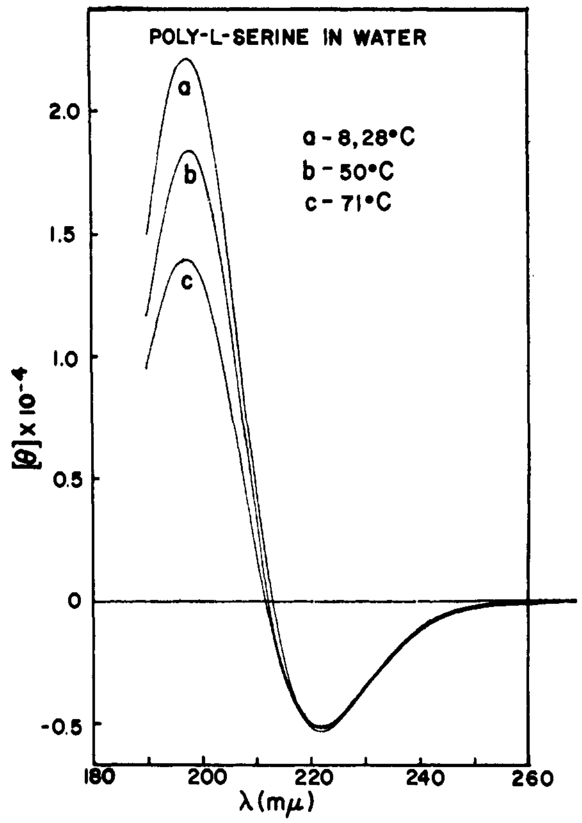

Figure 2. The temperature effect on the circular dichroism of poly-L-serine in water. It should be noted that the negative band does not change with increasing temperature, whereas the positive band is seen to decrease by more than 30% on raising the temperature from 28 to 71°. In water the transition begins at about 30°.

spectrum of poly-L-serine dissolved in 8 M LiCl would be that of a disordered form. The 200-mμ extremum resembles that obtained with random poly-L-glutamic acid or poly-L-lysine.[22] The presence of a weak negative band around 220 mμ compared with a positive one found at 218 mμ for random-coil polypeptides in aqueous solution may be due to a specific interaction of Li$^+$ with the peptide group or it may be that poly-L-serine has ϕ and ψ angles in the coil form which result in a negative n–π^* band.

The CD spectrum of poly-L-serine dissolved in water is similar to that obtained for polymers in the β conformation and is distinguished from the helical CD curves by the presence of only one negative extrema and of the positive extremum at 197 mμ rather than 191 mμ. Infrared data on solutions of PLS in D$_2$O in the region from 1600 to 1700 cm^{-1} show a band at 1620 cm^{-1} with a shoulder at 1650 cm^{-1}. This is what is to be expected from a mixture of β structure and random coil.[23,24] The rate of interconversion from random coil to β structure is enhanced by the presence in the solvent of large amounts of organic solvents such as trifluoroethanol (TFE), methanol, dioxane, and acetonitrile. The higher the mole per cent of organic solvent in the mixture, the faster the enhancement of the β-type CD curve. Representative CD curves obtained after equilibrium had been established are given in Figure 1. The magnitude of the positive band in the case of 80:20 (organic solvent:water) mixtures suggests that poly-L-serine is largely in the β conformation with little or no polymer in the random conformation. In water the amplitudes of the bands are much lower. Either a substantial per cent of random coil is still present in water after the equilibrium has been established or there is a direct effect of the solvent on rotational strengths. The data reported in Figure 1 for poly-L-serine are in qualitative agreement with the values reported by Iizuka and Yang[16] for *Bombyx morii* silk fibroin. The positive band, however, is greater for poly-L-serine. On the basis of the CD pattern and the ir data on poly-L-serine in solution and the ir and X-ray data on poly-L-serine and its derivative in the solid state, we assume poly-L-serine to be primarily in the β conformation in solution.

Temperature studies have been carried out on PLS dissolved in water and in water–TFE (20:80 v/v). The results are shown in Figures 2 and 3. Both solutions show a destabilization of the β structure with increasing temperature. The structure in water–TFE is more stable than in the aqueous solution. Cooling of the solutions previously maintained at high temperature does not result in immediate return to the ordered structure. However, leaving the solutions at room temperature for a sufficient period of time results in a return to the original CD spectrum. In the case of the aqueous sample the negative band did not show the temperature effect displayed by the positive band. The differential behavior of the 222- and 197-mμ bands upon heating in

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water would not be expected if the 222-mμ band contained an exciton contribution. Because of the steep distance dependence for the rotational strength of an n–π^* transition,[25] it is to be expected that this band would be sensitive to its immediate environment. It would be sensitive to specific solvent effects which may alter the orientation of the R group or in which a solvent molecule may itself act as a vicinal group. Perhaps it is with these considerations of the rotational strength of an n–π^* transition that the temperature effects shown in Figures 2 and 3 may be understood.

In regard to contributions to the rotational strength of the long-wavelength ellipticity band of the peptide chromophore, it is significant to note that the corresponding band in L-5-hydroxymethylpyrrolid-2-one has a molar ellipticity of -3.2×10^3 in trifluoroethanol with an approximate 2×10^{-40} for the rotational strength.[26] The vicinal perturbation giving rise to optical activity in this model compound is an hydroxymethyl group, *i.e.*, the same as the R group in poly-L-serine. The distances of the R groups on the same residue n and residue $n + 1$ in the polymer are closer than in the model compound. Accordingly one is forced to conclude that the R group perturbation is a major consideration in the polymer. Plots of the distance dependence for the rotational strength contribution of an hydroxymethyl vicinal group for a set of geometric factors are given elsewhere as is a more detailed consideration of the contributions of R groups to the rotational strength of the n–π^* transition in polypeptides.[26]

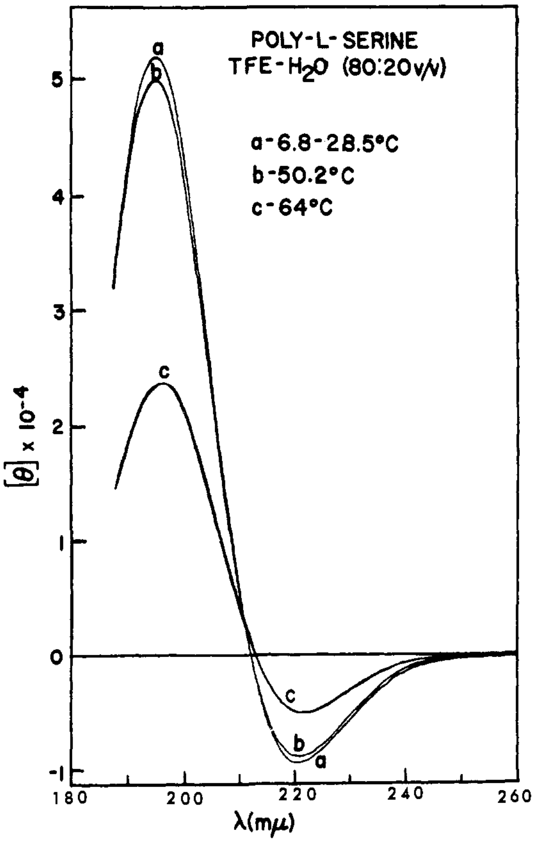

Figure 3. The temperature effect on the circular dichroism of poly-L-serine in 80% trifluoroethanol. In contrast to Figure 2, the negative and positive bands change proportionately with increasing temperature. In this solvent system the thermal transition begins at 50°.

Resolution of Absorption and Circular Dichroism Curves. The primary advantage of absorption and circular dichroism data on poly-L-serine is that characterization of this ordered homopolymer in terms of rotational strengths and dipole strengths is less ambiguous because of the absence of side-chain absorption bands in the wavelength range under study (260–190 mμ). Thus one can simultaneously fit the circular dichroism and absorption contours with a common set of Gaussian functions without having to make assumptions about the direct contributions of bands in the R group. In the absence of nonpeptide bands one has some assurance that a simultaneous fit of the two sets of data will aid in approaching a unique solution. As was suggested with poly-L-alanine relative to a helical polypeptide conformation, the rotational strengths and oscillator strengths of bands in poly-L-serine become a reference state for another ordered conformation (presumably a β structure) on which theoretical calculations may be based. A theoretical treatment capable of describing this relatively simple polypeptide may be used with less reservation to treat more complex protein systems.

Our approach to the resolution is to first fit the circular dichroism curve with a minimum number of Gaussian functions. In the case of poly-L-serine, two bands fit the circular dichroism data with such accuracy that there would appear to be no justification for considering additional bands. These two bands are then used to attempt description of the absorption curve by changing only sign and magnitude but without changing the mean or bandwidth. As was the case with poly-L-alanine[8] the long-wavelength CD band is at longer wavelengths than the absorption, and there is much excess absorbance at shorter wavelengths. The constraints of a common wavelength and bandwidth were similarly relaxed for the long-wavelength band of poly-L-serine. It was found that the absorption band was much broader and blue shifted when compared to the ellipticity band. At short wavelengths a band was added in absorption. As the added absorption band could, without changing the other two bands, satisfactorily describe the absorption curve and as the fitting of the CD curve could not be improved over the already excellent fit with two bands, there was no basis with which to arrive at a rotational strength for the short-wavelength absorption. The resolved curves for poly-L-serine in 80% trifluoroethanol are seen in Figures 4 and 5, and the critical values are given in Table I.

A molar extinction coefficient of 600 for the 214-mμ absorption band is the order of magnitude that is to be expected for an n–π^* transition.[27] That such transitions would have ellipticity bands red shifted from broader absorption bands was predicted theoretically by Moffitt and Moscowitz.[28] The above correlations, the accuracy of a single Gaussian fit, and the absence of a temperature effect between 8 and 70°, for the long-wavelength extremum in aqueous solutions of poly-L-serine (see Figure 2) suggest that this band is due to a

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Table I. Critical Values for Resolved Gaussian Curves for Poly-L-serine

Wavelength of extremum	Molar extinction coefficient $\times 10^{-3}$	Oscillator strength	Dipole strength[b] $\times 10^{36}$	Molar ellipticity $\times 10^{-3}$	Rotational strength[c] $\times 10^{40}$	Anisotropy,[d,e] $\lvert R_i/D_i \rvert$, $\times 10^4$
214 (222)[a]	0.6	0.019	0.9	−9.9	−5.6	6.2
197	3.5	0.065	2.85	52	32	11.2
184	6.8	0.17	6.8	Small	Small	Small

[a] The wavelength 214 mμ is the position of the longest wavelength resolved band in absorption. This represents a relaxation of the constraints that the same Gaussian bands must simultaneously fit absorption and circular dichroism data by changing only the sign and magnitude of the resolved bands. [b] The absence of ellipticity corresponding to the 184-mμ band is inferred in that no optical activity is required to improve the resolution. [c] The dipole strength is calculated from the expression (A. Moscowitz, "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1961, p 150) $D_i = 1.63 \times 10^{-38}(\epsilon_i\Delta_i/\lambda_i)$ where ϵ_i is the molar extinction coefficient at the curve maximum, λ_i is the wavelength of the ith maximum, and Δ_i is the half-band width at ϵ_i/e. [d] The rotational strength is calculated from the expression $R_i = 1.23 \times 10^{-42}(\theta_i\Delta_i/\lambda_i)$, where θ_i is the molar ellipticity at the maximum of the resolved curve; Δ_i and λ_i are defined above. [e] The significance of anisotropy is discussed by W. J. Kauzmann, J. E. Walter, and H. Eyring, *Chem. Rev.*, **26**, 339 (1940).

magnetic transition, likely the n–π* transition. A band at the same wavelength in helical polypeptides has been assigned to the n–π* transition,[22] and application of the concept of partial molar rotatory powers would be in accord with the above assignment of an n–π* transition[26,29] but would not be in accord with what is to be expected for an n–σ* transition.[30,8] The oscillator strength of the 214-mμ band is lower than observed for the n–σ* band of formamide.[30] In this connection it may be noted that the resolution, by allowing the absorption band to be at shorter wavelengths than the ellipticity band, results in a high value for the oscillator strength of the long-wavelength band.

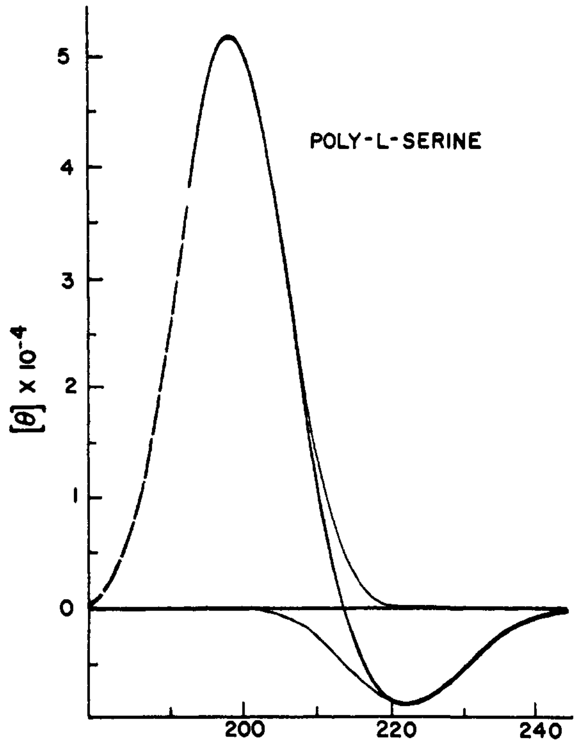

Figure 4. The resolved circular dichroism curve of poly-L-serine in 80% trifluoroethanol; see text for discussion.

The positive band at 198 mμ is in the proper wavelength range to be in the n–σ* transition,[31] and the oscillator strength is 0.065 which is only 10–15% greater than the calculated and experimental values for this band in formamide.[31] While the absorption curve blue shifts when trifluoroethanol is added to an aqueous solution (see Figure 6), the CD extremum exhibits either no shift or a slight red shift (see Figure 1). Also the sign of the band is what might be expected for an n–σ* transition.[30] Although the theoretical treatments have been in agreement with the assignment of the positive band to a transition arising from excitation resonance interactions between the monomer NV₁ transitions,[17,18] the possibility that this band is due to an n–σ* transition cannot, presently, be excluded.

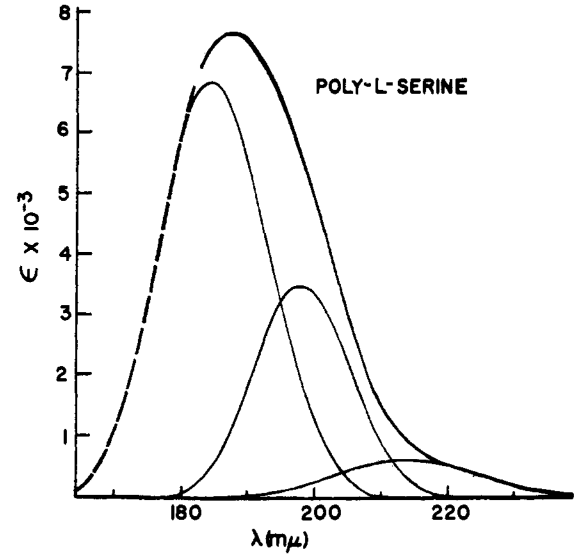

Figure 5. Resolved absorption curve of poly-L-serine in 80% trifluoroethanol; see text for discussion.

One of the interesting features of the resolution shown in Figures 4 and 5 is the excess absorbance at shorter wavelengths which is seen as an absorption band centered at 184 mμ with an oscillator strength of 0.17. In the Pysh calculations[18] a pair of bands of opposite signed rotational strength are observed at 195 and 198 mμ. These bands sum to give a net positive

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rotational strength. In such a situation, particularly when it is the relative position of bands and not their exact location which is of greater reliability, it is possible that a substantial absorption may be observed with little or no counterpart in the ellipticity curve. However, the Pysh calculations were for an infinite antiparallel pleated sheet whereas the degree of polymerization of the sample used in this study was 20. The calculations of Woody[32] are more relevant to the poly-L-serine data as they specifically treat the case of a cross-β or antiparallel pleated sheet structure containing 20 residues. In his results the NV_1 transition splits to give rise to a band at 202 mμ with a positive rotational strength, a pair of bands representing most of the absorption near 195 mμ with equal and opposite rotational strengths but with a splitting of only 1 mμ, and a band at 180 mμ, with a negative rotational strength. Allowing for expected error of several millimicrons in wavelength obtained from a theoretical treatment, the calculations very nicely explain the poly-L-serine data in that the major absorption exhibits little or no rotational strength and is at shorter wavelengths than the positive CD band.

Comments of the Variability of β-Type CD Patterns. Fasman and Potter have distinguished two β structures based on the positions of the optical rotatory dispersion extrema and crossovers.[33] Iizuka and Yang[16] find the positive CD extremum for silk fibroin at 197 mμ with a molar ellipticity of 3×10^4 and a negative band at 218 mμ with an ellipticity of less than 1×10^4, whereas Sarkar and Doty[14] and Townend, *et al.*,[15] report the negative band at 217 mμ and the positive band at 195 mμ with molar ellipticities of about 2×10^4 and $2\text{–}3 \times 10^4$, respectively. In the present communication on poly-L-serine we report the negative band at 222 mμ with a molar ellipticity of up to 1×10^4 and a band at 197 mμ with a molar ellipticity varying from 2 to 6×10^4. Poly-L-serine exhibits differential solvent and temperature effects (see Figures 1–3). Of some interest is the differential behavior with changes in temperature of the negative and positive bands of PLS in water. Temperature and solvent effects change absolute and relative magnitudes of the observed bands. It is apparent that there is such variability of the β-type CD curves, which are likely due to chain length, degree of chain association, and solvent effects, that one is presently precluded from assuming a given set of values to apply in a complex protein. Thus calculations of per cent β structure and of per cent α, β, and random coil in a protein, while of value, must be treated with due caution and skepticism. This reservation is further emphasized by the calculated chain-length effects for the β structure of poly-L-alanine.[29]

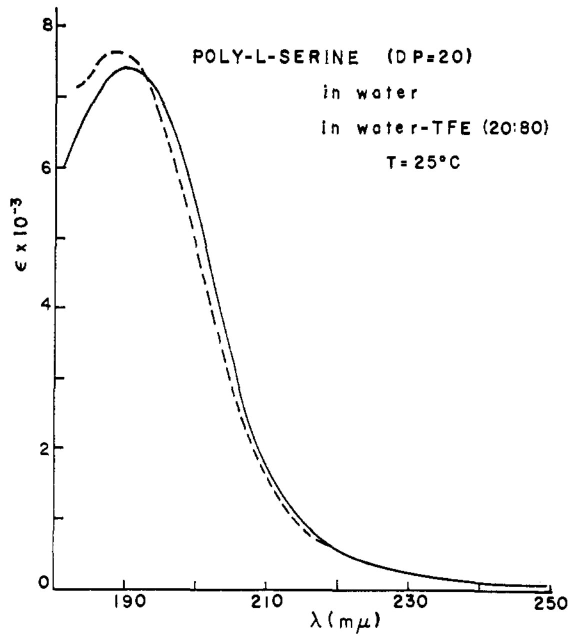

Figure 6. The effect of decreasing the dielectric constant of the medium. In 80% trifluoroethanol the major absorption peak is seen to be blue shifted at approximately 2 mμ.

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[32] R. W. Woody, private communication.

[33] G. D. Fasman and J. Potter, *Biochem. Biophys. Res. Commun.*, 27, 209 (1967).