Ultraviolet Rotatory Properties of Polypeptides in Solution. I. Helical Poly-L-alanine

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.
Received October 28, 1967

Abstract: Circular dichroism data are presented for helical poly-L-alanine, the simplest polypeptide in which the amino acid residue has intrinsic optical activity. Extrema are observed at 221, 207, and 191 mμ with molar ellipticities of -3.1×10^4, -3.6×10^4, and 6.6×10^4, respectively. The data are resolved into a set of component Gaussian curves. Resolved circular dichroism bands are centered at 221, 204, and 189 mμ with rotational strengths of -24×10^{-40}, -18×10^{-40}, and 71×10^{-40} and with dipole strengths of 0.58×10^{-36}, 0.71×10^{-36}, and 2.71×10^{-36}, respectively. The n–π* absorption band was found to be at shorter wavelengths and broader than the corresponding CD band in accord with the predictions of Moffitt and Moscowitz for magnetically allowed transitions. The data are discussed with respect to conformational, chain-length, R-group, and solvent effects.

In the last decade much work has been directed toward the development of optical rotation as a tool with which to study conformation of macromolecules in solution.[2,3] The impetus lies in the determination to describe biological molecules, *e.g.*, proteins and nucleic acids, in their functional states. The effort is redoubled as a result of much published data that demonstrate substantial changes in the optical rotatory dispersion (ORD) and circular dichroism (CD) patterns as the functional states of enzymes and other proteins are varied. The difficulties in properly utilizing these data arise from the complexities of optical rotation. A given electronic transition has many possible sources of rotational strength with the result of as many possible interpretations for a given set of data. This problem is often cloaked in the barren analysis that an increase in rotational strength of a chromophore may be interpreted as an increase in dissymmetry of the environment of the chromophoric group involved.

The complicated nature of optical rotation compounded by the complexity of proteins has naturally led to the study of model polypeptides and has led to work on rigid monomers.[4-6] Prominent among the polypeptide work is that of Holzwarth and Doty,[7] which demonstrated the presence of three bands in the ultraviolet CD spectrum of polypeptides: a negative n–π* band at 222 mμ, a negative band polarized parallel to the helix axis at 206 mμ, and a positive band polarized perpendicular to the helix axis at 190 mμ. This work was an essential step in providing basic data against which theoretical calculations could be compared. The polypeptides for which data were reported were poly-L-glutamic acid, poly-L-lysine, poly-γ-methyl-L-glutamate, and copoly-L-glu-lys-ala. As all of these polymers contained chromophores in the side chain with absorptions that overlap those of the peptide chromophore, the dipole strengths of the peptide transitions could not be reliably determined, and it had to be assumed that the side-chain transitions had no intrinsic rotational strength. Furthermore, theoretical treatments of the side chains as vicinal perturbations are complicated by lack of information of conformation of the side chain. These problems would be alleviated by data on poly-L-alanine. This report is the first of a series of papers which will present circular dichroism data on homopolymers and copolymers in the dissolved state. The first paper in the series reports data on poly-L-alanine which is the simplest optically active and helical polypeptide, the second treats poly-L-serine which gives a β-type CD curve and which has no side-chain transitions that would complicate interpretations of data to 185 mμ, and subsequent papers report data on homopolymers of the aromatic amino acids—histidine, tyrosine, and phenylalanine.

In order to more fully utilize optical rotation data on proteins, there are several aspects which must be clarified: (1) an experimental determination of the optical rotatory properties of basic polypeptide conformations, *e.g.*, α helix, 3_{10} helix, β structures, and random coils; (2) the effect of length of an ordered segment on the rotational strength and positions of the optically active transitions; (3) the effect of different vicinal perturbations arising from R groups containing different chromophores and conformations; (4) the effect of the medium (solvent effect) on the energies and rotational strengths of the peptide transitions.

This communication presents circular dichroism and absorption data on poly-L-alanine dissolved in trifluoroethanol containing a small amount of trifluoroacetic acid (0.75–1.5%). In addition, low molecular weight poly-L-alanine was studied in the absence of trifluoroacetic acid (TFA). The data were obtained on a CD unit of excellent specifications and on an absorption instrument capable of data to 170 mμ. In general the data were resolved with the requirement that the same set of Gaussian functions simultaneously fits both the CD and absorption curves by altering only

(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.
(2) (a) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961); (b) J. P. Carver, E. Shechter, and E. R. Blout, *J. Am. Chem. Soc.*, **88**, 2550 (1966).
(3) S. Beychok in "Poly-α-amino acids-Protein Models for Conformation Studies," G. D. Fasman, Ed., Marcel Dekker, Inc., New York, N. Y., 1967, Chapter 7.
(4) B. J. Litman and J. A. Schellman, *J. Phys. Chem.*, **69**, 978 (1965).
(5) F. A. Bovey, lecture presented at the International Symposium on Macromolecular Chemistry, Brussels, Belgium, June 13, 1967.
(6) D. W. Urry, *Ann. Rev. Phys. Chem.*, in press.
(7) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

the sign and the magnitude of the bands. These constraints were relaxed for the n–π^* transition in which case it was apparent that the CD band is at longer wavelengths than the absorption band and that the absorption band is broader. It was subsequently noted that these observations are in accord with the theoretical predictions of Moffitt and Moscowitz[8] for electrically forbidden and magnetically allowed transitions. Such critical comparisons were not possible with polypeptides containing chromophores in the side chains.

Previous work on poly-L-alanine was limited because of the means of solubilization, the solvents required, and the instrumentation employed. Solubilization had been achieved in chloroform or ethylene dichloride with an addition of strong organic acids such as trifluoroacetic acid or dichloroacetic acid.[9] Alternatively, solubilization had been achieved by coupling to water-soluble *dl* block polymers.[10,11] The organic solvent systems limited the ultraviolet penetration, whereas solubilization by block polymers resulted in large numbers of absorbing, nonrotating amino acids which decrease the reliability of measurements. The reported data were ORD measurements of limited uv penetration which are not as readily analyzed in terms of energies and rotational strengths of peptide transitions.

Experimental Section

Trifluoroethanol was a spectrograde product (Eastman Organic Chemicals). Trifluoroacetic acid (Eastman Organic Chemicals) was distilled over P_2O_5 at atmospheric pressure before use and stored in a drybox in an all-glass container. High molecular weight poly-L-alanine was obtained from Pilot Chemical Co. The sample gave a η_{sp}/c 0.87 when measured at c = 0.2 g/dl in TFA (25°) corresponding to a DP of about 600. Its degree of polymerization was estimated from correlation of random poly-γ-benzyl-L-glutamate viscosities with light scattering and sedimentation measurements.[12,13] Low molecular weight poly-L-alanine was obtained from Yeda (Rehovoth, Israel). Its molecular weight was reported by the manufacturer as 4000 (DP = 55). Before use, all polypeptide samples were dried overnight over P_2O_5 at 120° and stored in a desiccator over anhydrous calcium sulfate.

High molecular weight PLA was suspended in a 85:15 (v/v) mixture of TFE and TFA, respectively. In this mixture the sample is soluble to an extent of 2–3 mg/ml. This stock solution was passed through a sintered-glass filter and then diluted by a factor of 10 or more with TFE. The resulting solutions were then studied. The procedure of directly solubilizing PLA with a solvent mixture containing low amounts of TFA was discarded for the following reasons. The direct use of a low per cent TFA resulted in dilute solutions of PLA which would be difficult to accurately analyze for concentration. Therefore concentrations were determined before dilution by TFE. Moreover dilute TFA solvent mixtures might be expected to favor solubilization of low molecular weight PLA. With the procedure used no precipitation had occurred up to several weeks after dilution.

Concentrations of the stock solution were determined by dry weight after taking the solution to dryness. The stock solution was also analyzed in a Coleman 29 nitrogen analyzer. The concentrations determined by dry weight and by nitrogen content agreed to within 2%. Low molecular weight poly-L-alanine was suspended in pure TFE. The suspension was heated at 50° for 2 days. After passing through a sintered-glass filter the solution was concentrated in a rotating evaporator. The concentrated solution was then eluted through a column of Sephadex LH-20 for organic solvents. The column dimensions were 2.5 cm in diameter and 40 cm in length. The fractions were collected and their CD spectra run. Attempts to determine concentrations of these dilute fractions gave scattered results due to the limited amount of material available for each determination. The copolypeptide glu-ala-lys, 1:1:1, was a Pilot sample ([η] = 0.65 dl/g in water at pH 7.3). The sample was extensively dialyzed against water and then lyophilized.

Absorption and circular dichroism curves were obtained on a Cary Model 16 spectrophotometer and on a Model 6001 circular dichroism attachment for the Cary Model 60 spectropolarimeter. The CD unit was calibrated 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 at 290 mμ. The calibration was checked at shorter wavelengths using a solution of poly-L-glutamic acid at pH 4.2. Tests of absorption artifacts showed that an absorbance of 1.7 resulted in less than 1-mdeg variation in the CD curve of camphorsulfonate. The peak to peak amplitude of the curve herein reported was approximately 100 mdeg and the absorbance less than 0.5 at 187 mμ. Cell path lengths of 0.2 or 0.5 mm were used and were calibrated with chromate dissolved in 0.05 *N* KOH.

The absorption measurements were obtained with an instrument which has a negligible stray light level down to 175 mμ. Nitrogen was flushed through the instruments throughout both CD and absorption measurements. The temperature was kept at 25° unless otherwise stated. Sample temperatures were maintained with the Haake KT-62 Kryothermat and were monitored with a YSI Model 42SC telethermometer while spectra were being run. The resolution of the curves was performed with a Du Pont 310 curve resolver.

Results and Discussion

Before presenting the data it is pertinent to comment on dissolution of polypeptides using small amounts of strong organic acids. Straightforward interpretations (based on simpler amide systems) of infrared data on polypeptide solutions containing small amounts of organic acids led to the suggestion that the amide groups were protonated.[14–17] This interpretation has been shown to be incorrect, prior to the order–disorder transition, for several polypeptides on the basis that protonation of the acyl oxygen should affect the energy of the n–π^* transition of the peptide group.[18,19] Nuclear magnetic resonance studies on polypeptides in solutions containing strong organic acids also discount protonation.[20,21] Recent nmr and CD measurements on model compounds in the presence of TFA confirmed the absence of protonation by organic acids.[5] As addition of strong organic acids leads to an order–disorder transition, it is necessary to determine the conformational purity of poly-L-alanine under the conditions of characterization, *i.e.*, less than 1.5% TFA in TFE. CD spectra of solutions of PLA at the same concentration but in solvent mixtures containing increasing amounts of TFA showed no variation on the amplitude of the 220-, 207-, and 191-mμ bands until a concentration of 10–15% TFA is reached. This demonstrates that PLA in TFE with few per cent of TFA is in a fully ordered state.

(8) W. Moffitt and A. Moscowitz, *J. Chem. Phys.*, **30**, 648 (1959).
(9) G. D. Fasman in "Polyamino Acids, Polypeptides, and Proteins," M. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 221.
(10) W. B. Gratzer and P. Doty, *J. Am. Chem. Soc.*, 85, 1193 (1963).
(11) N. Lotan, A. Berger, E. Katchalski, R. T. Ingwall, and H. A. Scheraga, *Biopolymers*, **4**, 239 (1966).
(12) P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, 78, 947 (1956).
(13) J. C. Mitchell, A. E. Woodward, and P. Doty, *ibid.*, **79**, 3955 (1957).
(14) S. Hanlon, S. F. Russo, and I. M. Klotz, *ibid.*, **85**, 2024 (1963).
(15) S. Hanlon and I. M. Klotz, *Biochemistry*, **4**, 37 (1965).
(16) M. A. Stake and I. M. Klotz, *ibid.*, **5**, 1726 (1966).
(17) S. Hanlon, *ibid.*, **5**, 2049 (1966).
(18) F. Quadrifoglio and D. W. Urry, *J. Phys. Chem.*, 71, 2364 (1967).
(19) F. Quadrifoglio and D. W. Urry, in preparation.
(20) W. E. Stewart, L. Mandelkern, and R. E. Glick, *Biochemistry*, **6**, 150 (1967).
(21) J. A. Ferretti, *Chem. Commun.*, in press.

CD spectra were also recorded on solutions of low molecular weight PLA which were dissolved directly in TFE and eluted through a Sephadex column for organic solvents. Several fractions were collected and analyzed. Though it was not possible to accurately determine concentrations and thereby quantitatively compare PLA data in the presence and absence of TFA, it could be shown that the CD spectra of higher molecular weight fractions in pure TFE agreed qualitatively with the CD spectrum obtained in the presence of TFA. Thus addition of TFA was necessary only to achieve high enough concentrations to accurately quantitate the data. The CD curve of poly-L-alanine in TFE is given in Figure 1, whereas the curve in Figure 2 was determined with a small amount of TFA present.

Poly-L-alanine has an α-helical conformation in the solid state[22,23] as established by X-ray analysis. ORD on poly-L-alanine dissolved in $CHCl_3$–TFA or dissolved in water as a block copolymer[10,11] showed features common to other polypeptides known to be in α-helical conformations Our CD data on PLA dissolved in TFE–TFA confirm these suggestions. However, it should be emphasized that there is as yet no experimental basis with which to distinguish between α-helical and 3_{10} conformations. Because of this fact, in the present paper we shall refer to helical-type spectra without specific reference to the α helix. It may be noted in this regard that there are two types of helical CD spectra (see Figure 1). There is the type of CD spectrum observed in poly-L-lysine, myoglobin, and hemoglobin in which the extremum at 222 mμ due to the n–π^* transition is greater than that of the parallel band near 208 mμ. The second-type spectrum is found in poly-γ-methyl-L-glutamate, poly-L-alanine, and lysozyme which is noted by an inversion in the $[\theta]_{222}:[\theta]_{208}$ ratio; that is, the extremum due to the parallel band is substantially greater than that of the n–π^* band. The presence of 3_{10} helix in lysozyme[24] raises the possibility that the inversion be related to a conformational difference, though in the case of the homopolymers it may just as readily be a solvent effect. Both homopolymers were studied in TFE, and a terpolymer, glu-lys-ala, has been found to exhibit qualitatively similar changes on going from an aqueous solution to a trifluoroethanol solution (see below).

Resolution of Absorption and Circular Dichroism Curves. In order to fully utilize spectral data, it is necessary to resolve the complex curves into simple bands which may be assigned to specific electronic transitions. Once the electronic transitions have been characterized in terms of dipole strengths, rotational strengths, and anisotropies, the results may be compared with those expected from theoretical calculations. Such a set of values becomes a standard against which calculations are judged. A theory of the optical rotation of helical polypeptides which is capable of describing the simplest helical polypeptide can, with less reservations, be applied to considerations of the conformational changes observed in the more complex proteins. Data on

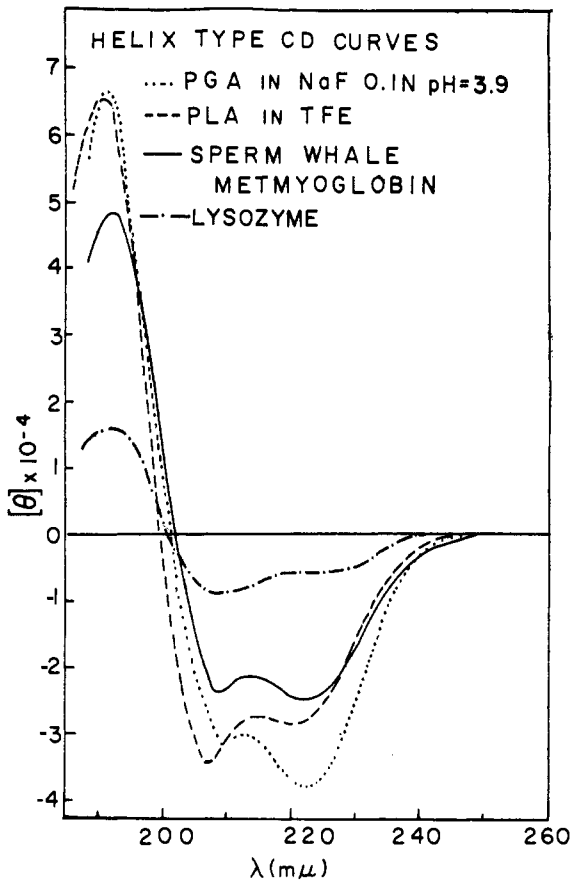

Figure 1. CD spectra of sperm whale myoglobin, poly-L-glutamic acid, low molecular weight poly-L-alanine, and lysozyme. The uncertainty of poly-L-alanine values is ±15% (see text).

poly-L-alanine are particularly significant as it is the simplest, optically active and helical polypeptide. The methyl R group is the simplest substituent which can impart intrinsic optical activity to the residue; its conformation is known, and it contains no electronic transitions at wavelengths longer than 160 mμ. Thus the bands resolved from the CD and absorption data of poly-L-alanine are, without ambiguity, those of the peptide chromophore, and the critical values may be considered without the reservations attending resolution of polypeptides with interfering chromophores in the side chain.

The problem of resolving a complex curve into a set of Gaussian functions lies in the attempt to obtain a unique solution. However, a unique solution cannot be claimed and in this resolution an obvious minimum number of Gaussian functions will be used. Clearly the CD curve of poly-L-alanine (see Figure 1) requires a minimum of three Gaussians in the 185- to 240-mμ wavelength range. Our approach to the resolution (using the Du Pont 310 curve resolver) was to first fit the circular dichroism curve. Then, without varying the width or position of the Gaussians, an attempt was made to fit the absorption curve by displaying the functions in the positive mode and by changing only the height of the function. The absorption curve fit was improved by slight changes in band width and position, and this set of curves was used to again describe the CD data. This iterative process was continued until a common set of bands could approximate both

(22) A. Elliot and B. R. Malcolm, *Proc. Roy. Soc.* (London), **A249**, 30 (1959).
(23) S. Armott and A. J. Wonacott, *J. Mol. Biol.*, **21**, 371 (1966).
(24) D. C. Phillips, presented at the 152nd National Meeting of the American Chemical Society, New York, N. Y., April 1967; *Sci. Am.*, **215**, 78 (1966).

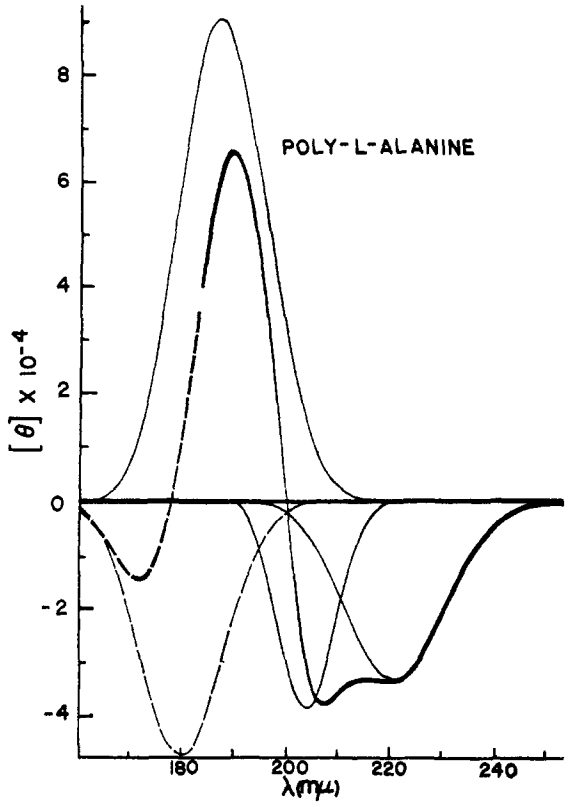

Figure 2. Resolved CD spectrum for poly-L-alanine in TFE–TFA (98.5:1.5 v/v). Bold-faced curve represents experimental data.

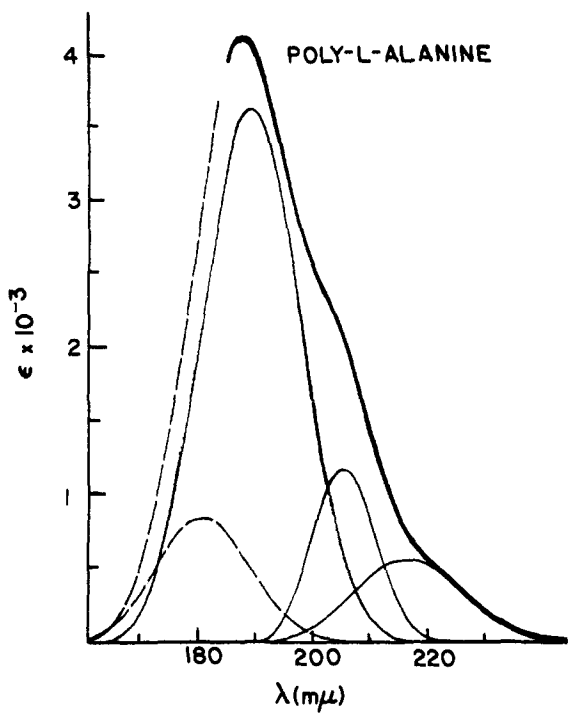

Figure 3. Resolved uv spectrum for poly-L-alanine in TFE–TFA (98.5:1.5 v/v). Bold-faced curve represents experimental data.

CD and absorption curves by changing only magnitude and sign.

The resulting fit was grossly inadequate both at the long-wavelength and short-wavelength sides of the complex curve. At the long-wavelength side it was apparent that the CD band should be at longer wavelengths than the absorption band, and at the short-wavelength side the absorption extremum was at shorter wavelengths than the CD extremum. Recognizing that the CD and absorption bands for an n–π^* transition need not be at the same wavelength, the restriction of position was relaxed for the long-wavelength band, and it was seen that the fit could also be improved if the constraint of a common band width were removed. At the short-wavelength side, an additional band was added to the absorption curve. In the ensuing iterative process, it was apparent that the best common fit could be obtained if the short-wavelength absorption band were also used as a negative CD band.

The resulting resolution is given in Figures 2 and 3, and the critical values are contained in Table I. An immediate point of interest is that of the position and width of the long-wavelength band. It was found that the absorption band was at shorter wavelengths and was broader than the associated CD band. This is in accord with Moffitt and Moscowitz[8] treatment of electrically forbidden but magnetically allowed transitions in which they state that "... we may anticipate that optical activity may sometimes be centered about a wavelength as much as 100 Å to the red of the absorption maximum, although this is rather extreme," and that "... the band width of an ellipticity curve may be narrower than that of the weak absorption spectrum with which it is associated." As may be seen in Table I, the ellipticity curve is shifted 50 Å to the red of the associated absorption band. The effect of relaxing the constraint of a common mean, however, results in a larger oscillator strength (f_{216} = 0.013) for the transition. This value is six to seven times greater than observed for formamide in acetonitrile.[25] The intensity of a weak or forbidden transition is enhanced by vicinal perturbations present in a helical system composed of asymmetric subunits. The importance of vicinal perturbations in increasing dipole strength of forbidden transitions has recently been treated by Robinson.[26] The observation that absorption and ellipticity bands are displaced in a manner predicted from considerations of vibronic coupling[8] and that the intensity of the absorption band is markedly increased by vicinal perturbations suggests that the vicinal perturbations act in concert with the vibronic mechanism.

By comparing the polarized absorption spectra[27] with the resolved absorption and CD data of poly-γ-methyl-L-glutamate, Holzwarth and Doty[7] could assign the negative long-wavelength CD band to the n–π^* transition which was polarized perpendicular to the helix axis, the second negative band to the parallel component of the monomer NV_1 transition, and the positive band to the perpendicular component of the monomer transition. The latter two bands had been predicted in their major aspect by Moffitt.[28,29] The oscillator strengths for the resolved bands of poly-L-alanine are lower than observed for poly-γ-methyl-L-

(25) H. Basch, M. B. Robin, and N. A. Kuebler, *J. Chem. Phys.*, 47, 1201 (1967).
(26) G. W. Robinson, *ibid.*, 46, 572 (1967).
(27) W. B. Gratzer, G. M. Holzwarth, and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, 47, 1785 (1961).
(28) W. Moffitt, *J. Chem. Phys.*, 25, 467 (1956).
(29) W. Moffitt, *Proc. Natl. Acad. Sci. U. S.*, 42, 736 (1956).

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

Wavelength of extremum	Molar extinction coefficient	Oscillator strength	Dipole strength[c] × 10^{36}	Molar ellipticity × 10^{-3}	Rotational strength[d] × 10^{40}	Anisotropy,[e] $\lvert R_i/D_i \rvert$, × 10^4
216 (221)[a]	0.55	0.013	0.58	−33	−24	40
204	1.16	0.017	0.71	−38	−18	24
189	2.62	0.065	2.71	91	71	32
180[b]	0.84	0.018	0.78	−47	−33	40

[a] The wavelength 216 mμ is the position of the longest wavelength resolved band in absorption. This represents the only 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 existence of a 180-mμ band is only inferred in that it markedly facilitates and improves 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).

glutamate as is the ratio $f_{\parallel}/f_{\perp}$. This is likely due to the contributions of the ester group. The positions of the resolved parallel and positive perpendicular bands in PLA are blue shifted 2 and 1 mμ, respectively, from those reported for poly-γ-methyl-L-glutamate. This is likely due to the different constraints used in the resolution and due to the effect of nonpeptide absorptions. The magnitude of the rotational strengths is slightly higher (−24 *vs.* −22) for the n−π* transition and lower for the parallel band (−18 *vs.* −29) and the positive, perpendicular band (+71 *vs.* +81). As mentioned above, the data could be best fit by including a band at 180 mμ which was negative in the circular dichroism. This result is in accord with the recently published calculation of Woody and Tinoco.[30] The 180-mμ band is required in absorption and, if made negative in ellipticity, improves the fit of the CD curve; however, it should be considered with due skepticism. The reported solution could be further improved by a small negative CD band centered at 197 mμ. The location of the band is suggestive of the n−σ* transition recently reported by Robin's group.[25] It may be a result of the Gaussian assumption and does not have the sign expected if an octant rule is operative.[31]

Comments on Conformational, Chain-Length, R-Group, and Solvent Effects. It was noted in the introductory section that there are four variables which are understood with varying degrees of clarity. With regard to conformation, it would appear correct that one can distinguish between helical, β, and disordered structures, the helical structures containing experimentally discernible parallel and perpendicular bands. At this stage it is not certain that one can distinguish between various helical structures—α, 3_{10}, etc. It is reasonable to ask on the basis of lysozyme[24] whether the higher parallel band is indicative of threefold helical structures. The observation that changing the solvent of gramicidin S from water to trifluoroethanol enhances the parallel band[32] does not favor a solvent-induced conformational transition. With regard to chain-length effect, Woody and Tinoco have calculated that the negative bands would give less chain-length dependence.[30] This is qualitatively what is observed with gramicidin S[33] which could have chain lengths of no more than about four residues.[34] The positive band, however, does exhibit substantial chain-length dependence.[33,30]

Now that detailed data have been presented on poly-L-alanine, it becomes possible to quantitate R-group effects. It has long been recognized that different side chains can alter conformation, but for a helical conformation it is now possible to assess differences in contributions of R groups. As the n−π* transition has a steeper distance dependence,[35] it might be expected

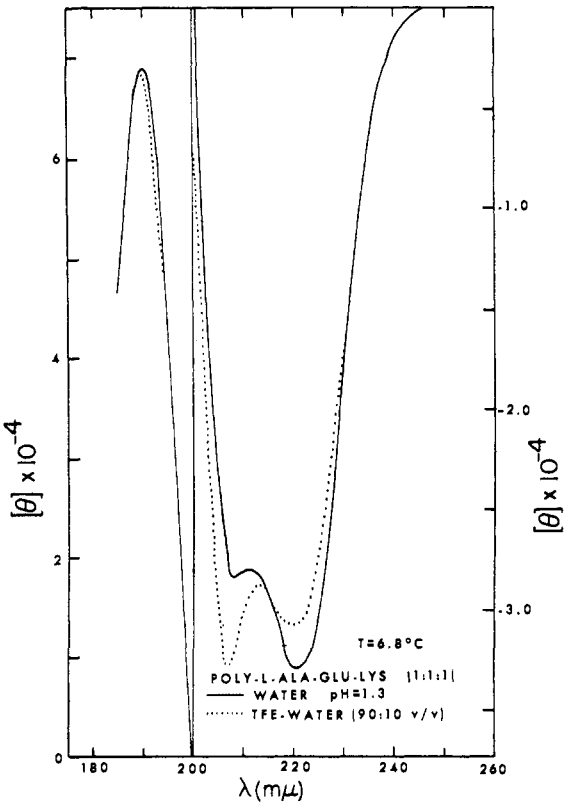

Figure 4. CD spectra for random copoly-glu-ala-lys (1:1:1) in water (pH 1.3) and in TFE–H_2O (90:10 v/v). As there is no change in the ellipticities between 6.8 and 20°, it would appear that the polymer is completely helical. Above 20° the helix–coil transition takes place. The right-hand ordinate is for the wavelength interval 200–260 mμ. The left-hand ordinate is for the shorter wavelengths.

(30) R. W. Woody and I. Tinoco, Jr., *J. Chem. Phys.*, **46**, 4927 (1967).
(31) D. J. Caldwell, *J. Phys. Chem.*, **71**, 1907 (1967).
(32) F. Quadrifoglio and D. W. Urry, unpublished data.
(33) F. Quadrifoglio and D. W. Urry, submitted for publication.
(34) A. M. Liquori, P. DeSantis, A. L. Kovacs, and L. Mazzarella, *Nature*, **211**, 1039 (1966).
(35) D. J. Caldwell and H. Eyring, *Rev. Mod. Phys.*, **35**, 377 (1963).

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.

(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).

(37) D. W. Urry and H. Eyring, *Perspectives Biol. Med.*, **9**, 450 (1966).

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.
Received November 9, 1967

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

(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.

(2) (a) J. C. Kendrew, *Science*, **139**, 1259 (1963); (b) M. F. Perutz, *J. Mol. Biol.*, **13**, 646 (1965).

(3) A. Elliot and B. R. Malcolm, *Proc. Roy. Soc.* (London), **A249**, 30 (1959).

(4) S. Armott and A. J. Wonacott, *J. Mol. Biol.*, **21**, 371 (1966).