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Computed Circular Dichroism Spectra for the Evaluation of Protein Conformation*

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ABSTRACT: Circular dichroism curves of poly-L-lysine containing varying amounts of α helix, β-pleated sheet, and random coil segments have been computed in the 190–250-mμ region. The application of these curves for determining protein conformation is discussed. The circular dichroism curves of several proteins, whose three-dimensional structures are known from X-ray diffraction studies, have been fitted by a linear combination of the three reference structures in the 208–240-mμ region. The results show that these computed curves are very useful in predicting protein structure, and if the protein possesses a high degree of secondary structure, the agreement between the calculated and the X-ray diffraction determined structure is extremely good. If the protein is largely nonregular, the results are less satisfactory but are still informative. The results show that the use of circular dichroism is a decided improvement over the use of optical rotatory dispersion for the evaluation of protein conformation.

In an attempt to evaluate the conformation of proteins we have previously reported computed optical rotatory dispersion curves for theoretical combinations of experimentally obtained curves for the α helix, the random coil, and the antiparallel pleated-sheet (β structure) conformations of poly-L-lysine (Greenfield *et al.*, 1967). These generated curves were compared with experimental spectra of lysozyme and myoglobin and it was found that curves which gave the best fit overestimated the β content and underestimated the α-helix composition, known from X-ray diffraction studies. The differences observed were attributed to aromatic side-chain chromophores, disulfide-bridge contributions, prosthetic group contributions, and possibly contributions from conformations of the amide bonds other than those in the three reference conformations. More recently Magar (1968) has used a more precise method of minimizing the variance between theoretical and experimental optical rotatory dispersion curves, but he essentially reached the same conclusions as Greenfield *et al.* (1967).

In this paper the computed spectra for conformational variation are reported for the circular dichroism spectra of poly-L-lysine based on the experimentally obtained curves for the three standard conformations: α, β, and random coil. These computed spectra are compared with the experimentally obtained spectra of several proteins whose conformations in the crystal state are known from X-ray diffraction studies. It was hoped that by using circular dichroism, which has less overlap of transitions than does optical rotatory dispersion, it would be possible to obtain closer fits of the spectra to those calculated from the crystal state conformations. The use of circular dichroism in the study of proteins has been recently reviewed by Beychok (1968).

Since our earlier optical rotatory dispersion study there have been several theoretical papers on the optical rotatory dispersion properties of polypeptides and simpler amides and there has been considerable doubt expressed that proteins can be analyzed adequately by use of only three parameters. Woody and Tinoco (1967) have calculated the theoretical optical activity of the α helix and observed that the rotatory strength of the π–π^* transition should be greatly dependent upon chain length. Scheraga's group (Vournakis *et al.*, 1968) and Tinoco *et al.* (1963) have calculated that the rotational strength of the n–π^* transition at 222 mμ of the α helix should also be chain length dependent. Urry (1968a) did not

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find such a chain-length dependence in his calculations on the rotatory strength of the n–π^* transition of the α helix, but did find an extremely large theoretical chain-length dependence for the n–π^* transition of the antiparallel pleated sheet (Urry, 1968b). The rotatory strength of the 3_{10} helix, recently found in proteins (Blake *et al.*, 1965), has also been shown to differ from that of the α helix and to display a chain-length dependence (Woody and Tinoco, 1967). Vournakis *et al.* (1968) have also calculated that nonaromatic side-chain effects will be found on the rotatory strength of the transitions in the helical form. The absolute values for different α helices have been experimentally found to vary (Adler *et al.*, 1968). Furthermore there is direct experimental evidence that polypeptides in the β form give several circular dichroism and optical rotatory dispersions patterns depending upon side chain and solvent (Fasman and Potter, 1967; Quadrifoglio and Urry, 1968; Tooney and Fasman, 1968; Stevens *et al.*, 1968). Sarkar and Doty (1966) moreover have shown that the β form of poly-L-lysine produced at neutral pH with SDS[1] has a slightly different spectrum than that found at pH 11 in water, and this has been confirmed by Li and Spector (1969). The magnitude of the circular dichroism ellipticity band at 218 mμ for this β form has only one-half the magnitude of the band of β poly-L-lysine produced by heating in water at pH 11.0, found by Townend *et al.* (1966) and by Sarkar and Doty (1966). Li and Spector state that β poly-L-lysine in water alone may form an intermolecular "infinite" pleated sheet and may not be a good model for the short sections of β structure found in proteins. Finally, it is not clear that the random coil of poly-L-lysine, an extended polyelectrolyte, is a good model of the "random" sections of proteins which have definite, but not repeating asymmetric structure. It has been shown that the random forms of poly-L-glutamic acid and poly(hydroxyethyl)-L-glutamine yield differences in their circular dichroism spectra, showing the effect of an extended *vs.* a contracted random form (Adler *et al.*, 1968). The circular dichroism spectrum of a film of random poly-L-lysine also displays distinct differences from that of random poly-L-lysine in solution, indicating that restricted random coils, as found in proteins, might yield other characteristic spectra (Stevens *et al.*, 1968).

Bearing all the above in mind, and being optimistic, we decided to try to correlate the circular dichroism spectra of poly-L-lysine with that of proteins and to see how satisfactory the correlation might be with the known conformations obtained from X-ray data. The results thus obtained justify this approach.

In the following work we make several assumptions which may not be fully justified. (1) That the circular dichroism of "infinite" poly-L-lysine (a homopolymer) is a good approximation of the circular dichroism spectra of short segments of a protein composed of different amino acids in the α-helical or β form. (2) That the random coil form of poly-L-lysine is a reasonable model for irregular sections of a protein, even though these are not truly random[2] but have a rigid, definite, nonrepeating structure. (3) That the 208–240-mμ region will be the best region to study. (The 208–240-mμ region was chosen, as the circular dichroism spectra of the three reference structures empirically seem less sensitive to nonchromophoric side-chain variation and solvent than the region below 208 mμ.) (4) That chromophores other than amides have minimal effect in this region.

TABLE I: The Circular Dichroism Spectra of Poly-L-lysine in the Three Reference Conformations.[a]

Wavelength (mμ)	[θ] in deg cm^2/dmole		
	α Helix[b]	β[c]	Random Coil[d]
190	74,800	22,400	−32,200
191	76,900 ± 8,400	25,300	−34,700
192.5	73,300	30,000	−37,500
195	64,300	31,900 ± 5,000	−41,000
197	44,300	30,000	−41,900 ± 4,000
200	14,300	24,300	−36,400
202	0	19,300	−25,600
205	−25,000	5,700	−14,500
208	−32,600 ± 4,000	−4,700	−3,400
210	−32,400	−10,800	−1,400
211	−32,100	−12,100	0
214	−31,000	−16,400	3,500
215	−31,400	−17,900	4,100
217	−33,100	−18,400 ± 1,800	4,600 ± 500
220	−35,300	−15,700	4,400
222	−35,700 ± 2,800	−13,800	3,900
225	−32,400	−11,400	2,700
230	−21,900	−6,400	800
234	−11,400	−3,600	0
238	−4,300	−1,400	−140
240	−3,300	700	−150
250	0	0	0

[a] Poly-L-lysine-HCl; intrinsic viscosity in 1 M NaCl, pH 4.0 = 1.67; estimated mol wt = 120,000. [b] Concentration = 0.01%; pH 11.1; 1-mm cell path length; temperature = 22°. Measurements at higher concentrations appear to yield higher values. It has previously been shown with poly-L-glutamic acid that there is a concentration dependence in measured rotation due to aggregation (Tomimatsu *et al.*, 1966; Yang, 1967; Adler *et al.*, 1968). [c] Same conditions as in footnote *b;* heated at 52° for 15 min; cooled to 22°. [d] Concentration = 0.01%; pH 5.7; cell path length 10 mm, 250–210 mμ; 1 mm, 210–190 mμ.

Experimental Procedure

Circular dichroism measurements of poly-L-lysine were obtained with a Cary 60 recording spectropolarimeter fitted with a Model 6001 circular dichroism attachment and set for a

[1] Abbreviation used is: SDS, sodium dodecyl sulfate.

[2] In this paper "random" is the term describing nonregular, nonrepeating structures in proteins. These structures are not truly random, as they have a fixed ordered structure. The term "random coil" is the term describing disordered polypeptides.

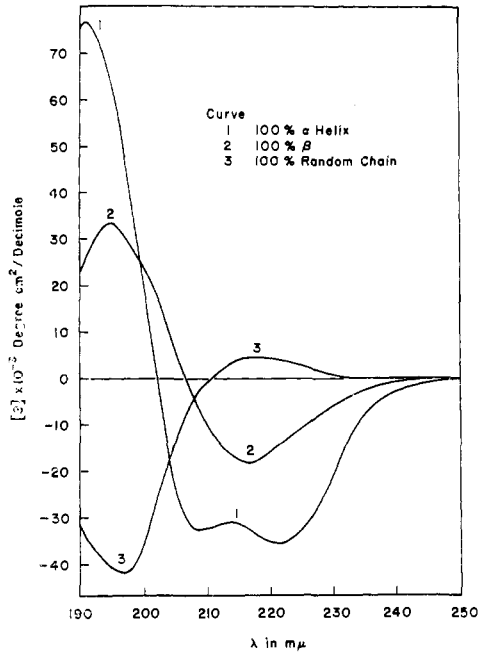

FIGURE 1: Circular dichroism spectra of poly-L-lysine in the α-helical, β, and random conformation.

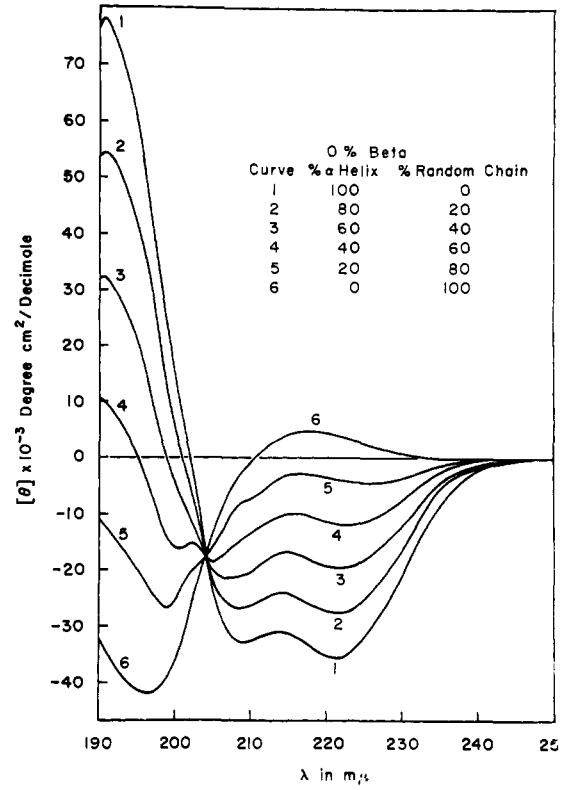

FIGURE 2: Calculated circular dichroism of poly-L-lysine containing 0% β and varying percentages of α helix and random coil, as indicated.

half-band width of 15 Å. Measurements were made at 22°. Preparation of the three forms of poly-L-lysine were performed as described in Greenfield *et al.* (1967), and studied under the conditions described in Table I. The circular dichroism spectra of poly-L-lysine in the three forms have been published previously by Townend *et al.* (1966) and by Sarkar and Doty (1966), and is in substantial agreement with the data reported herein. The data are shown in Table I.

Three-times-recrystallized lysozyme obtained from Sigma Chemical Corp. was studied in water at pH 6.6, as described by Greenfield *et al.* (1967), in the concentration range from 0.01 to 0.04% in a 1.0-mm cell. The concentration was determined using an $\epsilon_{1\,cm}^{1\%}$ of 26.5 (ϵ_m 38.2 × 10^3) (Steiner, 1964).

Chymotrypsin and chymotrypsinogen were identical with those used by Fasman *et al.* (1966) and were studied in 0.4 M NaF at pH 7.0 at concentrations of 0.02–0.1%, in path lengths of 0.2 and 1.0 mm. The concentration was determined for both using an $\epsilon_{282}^{1\%}$ of 20 (ϵ_M 5.0 × 10^4) (Wilcox *et al.*, 1957a,b).

Two-times-recrystallized carboxypeptidase A, obtained from Worthington Biochemical Corp., was studied in 0.02 M Na_2HPO_4–0.02 M NaF at a concentration of 0.07% in a 1-mm cell. The pH was adjusted to 7.6. The concentration was determined using an ϵ_M of 6.42 × 10^4 (Simpson *et al.*, 1963).

Circular dichroism results are reported in terms of [θ], the mean residue ellipticity in units of deg cm²/dmole.

In all the above proteins the mean residue weight was taken as 115.

Values for the ellipticity of RNase were obtained from curves of Timasheff and Stevens (1969). It was studied at pH 4.5 in water on a Jacso Model ORD/UV5. Values for the ellipticity of myoglobin at pH 7.0 were taken from the published curve of Urry (1968c). Values for the circular dichroism of β poly-L-lysine with SDS were taken from the curve published by Li and Spector (1969) and values for the circular dichroism of poly-L-serine in water were taken from graphs in the paper by Quadrifoglio and Urry (1968).

The calculation of theoretical combinations of α helix, β structure, and random forms of poly-L-lysine in water was performed on an IBM 1130 computer as described for the optical rotatory dispersion curves given in Greenfield *et al.* (1967). The values for proteins which were obtained experimentally were compared with theoretical curves *via* a procedure similar to that described by Magar (1968). Calculations were performed on a Wang Model 370 programmed calculator using programs for normalizing the data and solving simultaneous equations generously supplied by Dr. Kirk Aune. The variance between the experimental and theoretical combinations of α helix, β structure, and random coil were minimized from 208 to 240 mμ.

Results and Discussion

The circular dichroism curves for average values of the α helix, β, and random forms of poly-L-lysine are shown in Figure 1. Experimental values are listed in Table I. Examples of calculated percentages are shown in Figures 2–8. Examination of the curves in Figure 1 shows that both the β form and random coil have a very low ellipticity equal to approximately −4000 deg cm²/dmole at 208 mμ. The α helix has an extremum at this point equal to approximately −33,000 deg cm²/dmole. To a first approximation (method I) the per cent α helix can be calculated from

$$\%\ \alpha\ \text{helix} = \frac{[\theta]_{208\ m\mu} - 4{,}000}{33{,}000 - 4{,}000} \qquad (1)$$

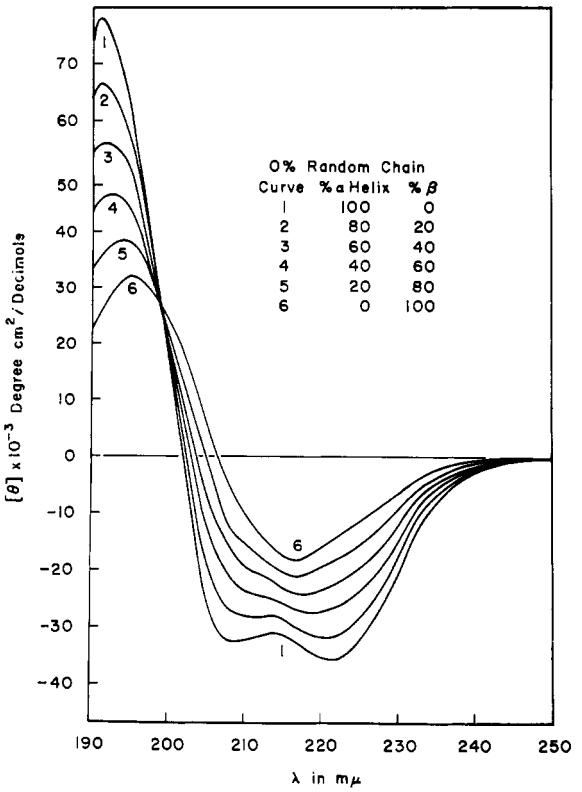

FIGURE 3: Calculated circular dichroism of poly-L-lysine containing 0% random coil and varying percentages of α helix and β structure, as indicated.

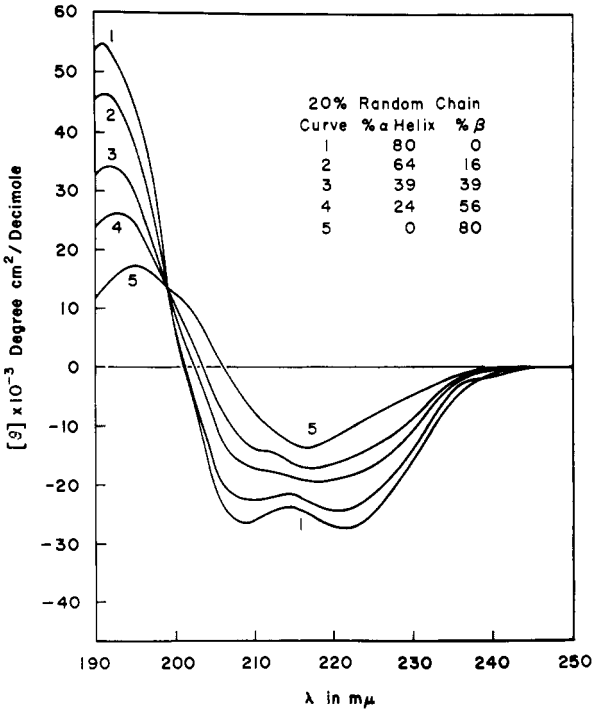

FIGURE 4: Calculated circular dichroism of poly-L-lysine containing 20% random coil and varying percentages of α helix and β structure, as indicated.

The value obtained for the per cent α helix would be only an estimate, as the value of the ellipticity of the α helix is somewhat chain length dependent (Tinoco *et al.*, 1963; Woody and Tinoco, 1967; Vournakis *et al.*, 1968). Upon examination of a set of curves with a fixed percentage of α helix, it is seen that the shape of the curve depends upon the amount of β structure and random coil present. An estimate of the percentage of random coil and β structure can thus be made from the shape of the curves, once the approximate percentage of α helix is determined from eq 1. A convenient method of finding the per cent β structure and random coil is to compare the values at 217 mμ (the extremum for β structure) and 222 mμ (the second extremum for the α helix) of the experimental curves with those of the curves shown in Figures 2–8. If the experimental values lie between values represented in Figures 2–8, the percentage of random coil can be estimated to within 10% by taking an average of the two closest curves illustrated; the remainder is then β. The data below 208 mμ has not been considered in the estimation of composition because the circular dichroism of the α helix would show the greatest chain-length dependence in this region (Woody and Tinoco, 1967), and the low-wavelength peak of the β form of polypeptides is greatly solvent dependent in this region (Quadrifoglio and Urry, 1968). Furthermore, aromatic groups have extremely high extinction coefficients below 200 mμ (Gratzer, 1968) and might have large circular dichroism bands as well. Also, the magnitude and position of the ellipticity bands of random coils vary considerably in this region (Stevens *et al.*, 1968; Adler *et al.*, 1968). On the other hand, the data in the 208–240-mμ region appear to be reliable, as Peggion *et al.* (1968) have shown that up to 16% tryptophan in helical copolymers of poly-L-tryptophan and poly-γ-ethyl-L-glutamate has no effect on the circular dichroism from 208 to 240 mμ. The magnitudes of the ellipticity of the β structure of various polypeptides are more similar in the 208–240-mμ region, and differences due to solvent are also smaller and should not introduce as great an error in

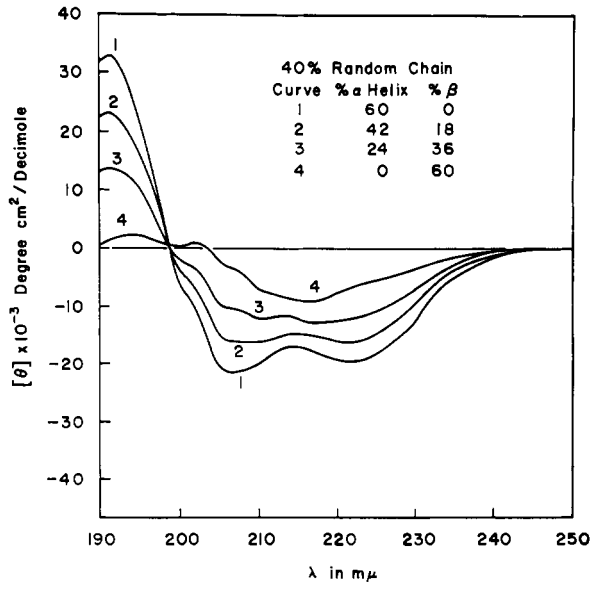

FIGURE 5: Calculated circular dichroism of poly-L-lysine containing 40% random coil and varying percentages of α helix and β structure, as indicated.

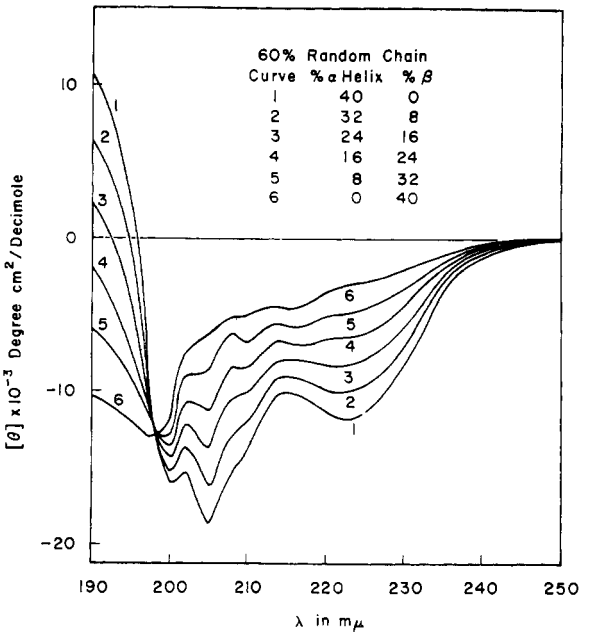

FIGURE 6: Calculated circular dichroism of poly-L-lysine containing 60% random coil and varying percentages of α helix and β structure, as indicated.

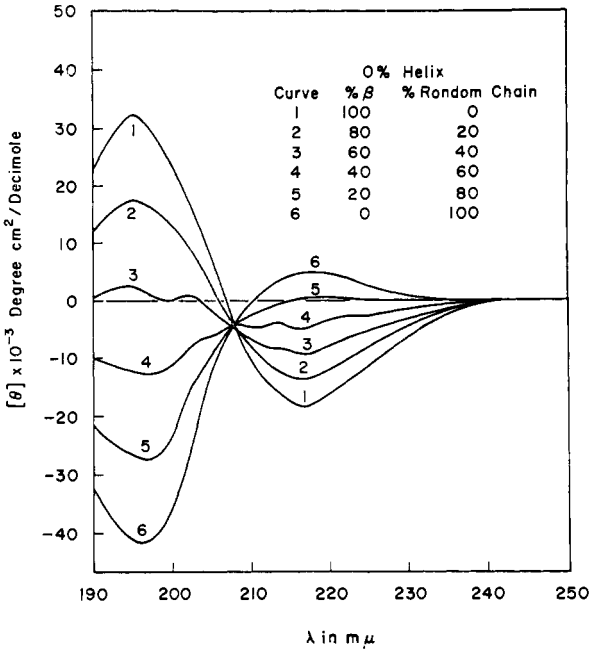

FIGURE 8: Calculated circular dichroism of poly-L-lysine containing 0% α helix and varying percentages of β structure and random coil, as indicated.

the calculation of the per cent α helix, β, and random coil, if only poly-L-lysine in water is used as the standard.

To a first approximation by using the procedure outlined above (method I), the following estimates of protein composition were obtained as shown in Table II. If the protein had a peak near 208 mμ its value rather than the value precisely at 208 mμ was used in eq 1. After the helical content was estimated, the structure was evaluated to within the nearest 10% of random coil. The results for the first three proteins are reasonable, while the last three deviate considerably from the known values.

To refine our estimates the variance was minimized between the experimental circular dichroism curves of several proteins and a linear combination of the circular dichroism curves for the α helix, β structure, and random coil between 208 and 240 mμ (method II). The values of α helix, β structure, and random coil used are shown in Figure 1. To investigate the differences in composition of proteins that might be

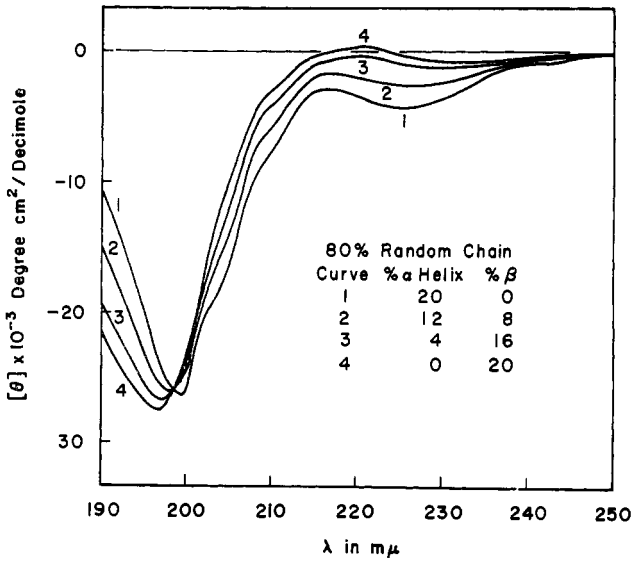

FIGURE 7: Calculated circular dichroism of poly-L-lysine containing 80% random coil and varying percentages of α helix and β structure, as indicated.

TABLE II: Estimated Percentages of α Helix, β Structure, and Random Coil of Proteins from Circular Dichroism Curves[a] by Method I.[b]

Protein	% α Helix	% β Structure	% Random Coil
Myoglobin	67	0–13	20–30
Lysozyme	29	11	60
RNase	12	38	50
Carboxypeptidase A	15	25	60
Chymotrypsin	12	18	70
Chymotrypsinogen	20	10	70

[a] See Table III for X-ray determined values. [b] Per cent α-helix is estimated by eq 1.

$$\% \alpha \text{ helix} = \frac{[\theta]_{208\ m\mu} - 4{,}000}{33{,}000 - 4{,}000}$$

Per cent random coil is estimated to within 10% by comparison with calculated curves at 218 and 222 mμ, and the remainder is taken as β structure.

TABLE III: Comparison of Conformation Obtained by Circular Dichroism and X-Ray Data by Method II.[a]

Protein	X-Ray Structure			References	Circular Dichroism Calcd Structure					
					α		β		Random Coil	
	α	β	Random Coil		b	c	b	c	b	c
Myoglobin	65–72, 77[d]	0	32–23	Kendrew *et al.* (1960)	68.3	68.2	4.7	7.9	27.0	23.9
Lysozyme	28–42[d]	10	62–48	Blake *et al.* (1965); Phillips (1966, 1967)	28.5	29.9	11.1	9.3	60.4	61.0
RNase	6–18[d]	36	58–46	Kartha *et al.* (1967)	9.3	12.0	32.6	43.4	58.1	44.5
RNase S	15[e]	31	54	Wyckoff *et al.* (1967)						
Carboxypeptidase A	23–30[d]	18	59–52	Reeke *et al.* (1967)	13.0	15.9	30.6	39.9	56.4	44.2
α-Chymotrypsin[f]	3[g]			Sigler *et al.* (1968)	11.8	13.4	22.8	31.9	65.5	54.8
Chymotrypsinogen					13.8	17.3	25.2	28.9	60.9	53.8

[a] The best fit to the experimental circular dichroic curve was found by minimizing the variance between the experimental circular dichroic curve and a linear combination of circular dichroic curves for the α helix, β structure, and random coil from 208 to 240 mμ. [b] Values used in calculations are for poly-L-lysine in H_2O, from Figure 1. [c] Values used in calculations same as in footnote *b* with data for β poly-L-lysine in SDS substituted for β poly-L-lysine in H_2O. [d] Lower value represents true regular α helix; upper value, total helix including 3_{10} and distorted helices. [e] Helical type not distinguished by authors. [f] α-Tosylchymotrypsin used for X-ray work. [g] Only one short-chain section α helix included; isolated helical turns and β structure not reported, although they may be present.

obtained by using different reference β structures, the ellipticity for β poly-L-lysine at neutral pH in SDS given by Li and Spector (1969) was used in place of the β form of poly-L-lysine at pH 11.0 in water. An attempt was also made to use the values for poly-L-serine in water (Quadrifoglio and Urry, 1968) as another β reference value. Unfortunately, this form has a negative ellipticity band at 222 mμ, is not distinguished by the minimization procedure from a combination of the α-helical and random forms, and thus was discarded. When a program utilizing four parameters was used (the α helix, β structure, and random coil of poly-L-lysine in water and the β form of poly-L-serine in water), the results were also not meaningful. For example, the coefficient of the β form of poly-L-serine was negative when the following equation was fitted to chymotrypsinogen. $CD_{protein} = a \times \alpha$-helix lysine $+ b \times \beta$-lysine $+ c \times$ random coil lysine $+ d \times \beta$ serine, where $a + b + c + d = 100\%$ (CD, circular dichroism). The data clearly were not resolvable by such a treatment.

The results of our treatment, compared with the structures obtained from X-ray crystallographic studies, are summarized in Table III. It is seen that when a protein has considerable α helix and β structure the results are within 5% of the X-ray data, and either value for the β form of poly-L-lysine (the value in water or in SDS) suffices to give a good estimate. Thus, the results with myoglobin, lysozyme, and RNase are quite good. These results are shown in Figure 9. The solid line represents the experimental curve and the points are the theoretical best approximation. As expected, one is able to obtain a good fit in the region of 208–240 mμ where the variance of the data is minimized, but the fit is very poor below 208 mμ. It is noted that the computed best fits are close to the approximations obtained by inspection (Table II, Method I). The disparity between the calculated and experimental curves below 208 mμ may stem from several facts. The contribution of the nonregular segments in proteins may be far less than found for highly charged random poly-L-lysine in this region (Adler *et al.*, 1968). The aromatic contributions may be considerably larger below 208 mμ than above (unpublished data). Solvent perturbations, which may mirror local environment effects, also are more important at lower wavelengths (Quadrifoglio and Urry, 1968).

When the proteins studied are largely "random,"[2] the results are not as good. Thus, the amount of helix is overestimated for both chymotrypsinogen and chymotrypsin, and underestimated for carboxypeptidase A. β structure is likewise overestimated for carboxypeptidase A. Furthermore, in the case of chymotrypsinogen, the calculated best fit is poor even in the 208–240-mμ region. These results are shown in Figure 10. The lack of agreement for these proteins is probably due to both chain-length and side-chain effects, discussed earlier. When the mean residue rotation of the protein is low due to a small amount of periodic secondary structure or cancellation of β and random contributions, the side-chain effects, of course, have greater importance in the total circular dichroism spectrum of the protein. However, even in the less satisfactory examples, the results give a fair approximation to the structure of the proteins.

It is satisfying to note also that, even though there is apparently a large difference between the circular dichroism spectrum of chymotrypsin and that of chymotrypsinogen, the calculated structure for each protein is very similar. As the computed curves are similar, perhaps the deviations in the experimental curves would indicate differences due to side-chain orientation. The lack of gross change of secondary structure upon activation of chymotrypsinogen to chymotrypsin is in accord with the interpretation from X-ray diffraction studies of Reeke *et al.* (1967).

It is pertinent to ask how useful is this method of analyzing circular dichroism data of proteins compared with earlier

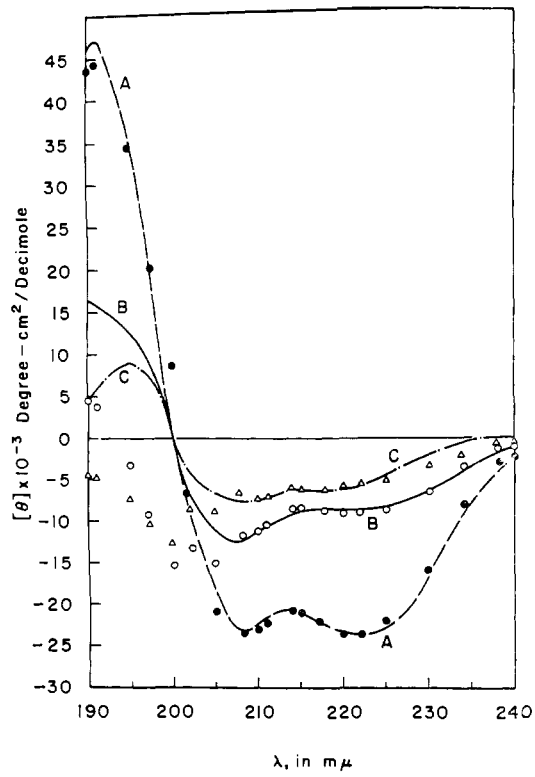

FIGURE 9: The circular dichroism of: (A) myoglobin (curve) and 68.3% α helix, 4.7% β structure, and 27.0% random coil, calculated from poly-L-lysine reference spectra in water (●●●●). (B) Lysozyme (curve) and 28.5% α helix, 11.1% β structure, and 60.4% random coil (○○○○), calculated as in part A. (C) RNase (curve) and 9.3% α helix, 32.6% β structure, and 58.1% random coil, calculated as in part A (△△△△).

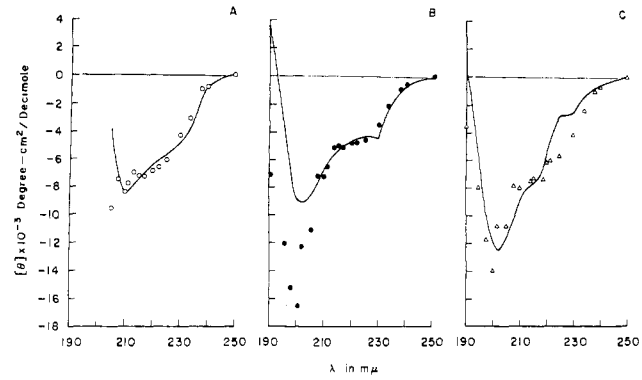

FIGURE 10: The circular dichroism of: (A) carboxypeptidase A (solid curve) and 13% α helix, 30.6% β structure, and 56.4% random coil, calculated from poly-L-lysine spectra in water (○○○○). (B) α-Chymotrypsin (solid curve), and 11.8% α helix, 22.8% β structure and 65.5% random coil, calculated as in part A (●●●●). (C) chymotrypsinogen and 13.8% α helix, 25.2 β structure, and 60.9% random coil, calculated as in part A (△△△△).

methods of estimating protein structure from optical rotatory dispersion. A review of these methods has appeared in Greenfield *et al.* (1967). The answer is encouraging. For those proteins in which the curve approximation is poor, the circular dichroism method is at least no worse than any previous method used. For those proteins for which the fit is good, the results equal or surpass previous methods.

Most early methods estimated only helical content, and in this task the use of circular dichroism in wavelength region 208–240-mμ is equal to earlier methods. Moreover, circular dichroism is superior to those methods using optical rotatory dispersion which attempted to estimate also β structure.

For myoglobin, the best fit of the circular dichroism spectrum from 240–208 mμ gives a helical content of 68%. This is equal to the value of 65–71% obtained from b_0[3] of the Moffitt equation (Urnes *et al.*, 1961; Harrison and Blout, 1965) and definitely superior to the value of 56% obtained from the trough at 233 mμ (Breslow *et al.*, 1965), and the best fit to the optical rotatory dispersion from 250 to 190 mμ (Greenfield *et al.*, 1967; Magar, 1968) which gives a helical content of 55% and overestimates the amount of β structure by 35%.

For lysozyme, the closest circular dichroism approximation from 240 to 208 mμ gave a helical content of 29%, which was consistent with values of 30–33% obtained from the Moffitt equation (Doty, 1957; Tomimatsu and Gaffield, 1965), 32% calculated from the Shechter–Blout equation (Tomimatsu and Gaffield, 1965). Furthermore, the analysis of the circular dichroism spectrum gave a value for β structure of 11%, which is consistent with the X-ray data. Previous attempts using optical rotatory dispersion from 250 to 190 mμ (Greenfield *et al.*, 1967; Magar, 1968) or optical rotatory dispersion in the visible region using the constants a_0 and b_0 of the Moffitt equation (Troitski, 1965) gave results which seriously overestimated β structure. The far-ultraviolet optical rotatory dispersion of lysozyme gave α-helical contents of 20–21% and β contents of 32–35%. The Troitski (1965) analysis of the near-ultraviolet data gave values of 36% α helix and 17% β structure.

For RNase the circular dichroism results are also superior to methods utilizing optical rotatory dispersion data. The values of α helix and β structure obtained from the circular dichroism data are 9 and 33%, respectively, which agree well with the X-ray structure data. The helical content of 17% obtained from the b_0[3] value of the Moffitt equation is also in agreement with the X-ray data (Doty, 1957). The calculated value of 26–27% from the Shechter–Blout equation (Shechter and Blout, 1964) and the value from the trough at 228 mμ (Cathou *et al.*, 1965), however, both overestimate helical content. The procedure of Troitski (1965) overestimates α helix at 21% and greatly underestimates β structure at 10%. The circular dichroism spectrum from 208 to 240 mμ, furthermore, can be analyzed in terms of α helix and β structure without postulating any shifts in the energy of the n–π^* transition of the α helix as proposed by Schellman and Lowe (1968) to justify the optical rotatory dispersion trough at 228 mμ. The circular dichroism data of Tamburro *et al.* (1968) was also interpreted in terms of α, β, and random structure. The analysis of RNase by the closest circular dichroism approximation from 240 to 208 mμ is in agreement with the analysis of RNase by S. Beychok (personal communication, 1969). The deviation between the calculated and observed spectra for RNase above 230 mμ may be due to aromatic

[3] The helical content was calculated using $b_0 = -600$ for 100% helix.

bands or bands from the disulfide bridges in the molecule; however, they do not interfere with obtaining a good approximation.

The best accommodation to the circular dichroism spectrum of carboxypeptidase A is not as good as those for the three previous proteins, but it is as good as previous methods. The Moffitt equation gave values for the α helix ranging from 20 to 40% (Lipscomb *et al.*, 1966; Quiocho *et al.*, 1967). The trough in the optical rotatory dispersion of carboxypeptidase A is shifted to 236 mμ and has a low rotation $[m']$ = 2700 deg cm^2/dmole (Quiocho *et al.*, 1967), which indicates a very low-helical content. Troitski's method (1965) overestimates helical content at 40% and β content at 31%. The best fit of the optical rotatory dispersion from 250 to 190 mμ has no helix at all and a very large β content of 65% (N. Greenfield, J. Potter, and G. D. Fasman, unpublished data).

For chymotrypsin (12% α, 23% β) and chymotrypsinogen (14% α, 25% β) the results are again no worse than previous methods. The b_0^3 value of the Moffitt equation for chymotrypsin in 0.05 M PO_4–0.1 M β-phenyl propionate would indicate an α-helix content of 23%, the b_0^3 of chymotrypsinogen under the same conditions gives a helical content of 12% (Imahori *et al.*, 1960). Raval and Schellman (1965) found a helical content from the Moffitt equation and from the 233-mμ trough of the optical rotatory dispersion ranging from 12 to 15%. Biltonen *et al.* (1965) found the same value. These authors feel that changes which are noted in the optical rotatory dispersion upon activation are due to side-chain chromophores. Fasman *et al.* (1966) also noted changes in side-chain chromophores but could not rule out small changes in the helical content as well. The results of Raval and Schellman (1965) and Biltonen *et al.* (1965) are in agreement with the results here.

In conclusion, the use of circular dichroism does appear to be a superior method for determining protein conformation than optical rotatory dispersion. A good estimate of protein structure can be obtained from the computed curves based on the α, β, and random conformations of poly-L-lysine, even though there are many factors that may contribute to the circular dichroism other than the three reference structures chosen. The estimation of conformation of proteins will probably improve when the contributions of the side-chain chromophores and disulfide bridges are better delineated.

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The Single-Stranded Polyadenylic Acid–Poly-L-lysine Complex. A Conformational Study and Characterization*

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ABSTRACT: The effect of poly-L-lysine on the conformation of single-stranded polyadenylic acid has been studied by optical rotatory dispersion and ultraviolet spectroscopy. The ultraviolet and optical rotatory dispersion spectra of polyadenylic acid are altered on addition of poly-L-lysine at pH 7.0. The ultraviolet maximum of 256 mμ for polyadenylic acid shifts to 262 mμ for the complex. The optical rotatory dispersion spectrum is also red shifted (4 mμ), and decreased in molar rotation ($[m]_{256}^{\text{poly A}} = -78{,}000$, $[m]_{260}^{\text{complex}} = -23{,}000$) and complexity as compared with polyadenylic acid. Complex formation occurs in two steps. The primary interaction involves single-chain association, which is followed by a secondary aggregative interaction. The primary association occurs with a 1:1 residue stoichiometry, a large association constant, relatively little insolubility, and an apparent insensitivity to both the degree of charge on the poly-L-lysine and to ionic strength (100–40% charge, H_2O to 0.2 M NaF). The secondary (aggregative) interaction, which occurs near residue equivalence, is marked by further red shifts of the optical rotatory spectra and increased insolubility. Aggregation occurs maximally when the poly-L-lysine is 50% helical. There is no complex formation in 0.2 M NaF when the poly-L-lysine has zero charge and is 100% helical.

It is concluded that poly-L-lysine forms a well-defined complex with polyadenylic acid, altering the conformation of the latter.

Interest in the mechanism of cellular differentiation has prompted extensive research, some of which considers the possible role of histones in gene expression. One proposal suggests that these basic proteins exert control by altering the physical character of that portion of the chromosome to which they are bound, thus affecting its properties as a template for RNA synthesis (Stedman and Stedman, 1950; Ts'o and Bonner, 1964).

Previous work has shown that nucleic acids and synthetic polynucleotides form complexes *in vitro* with a variety of cationic molecular species ranging in size from Mg^{2+} ion through diamines such as spermine and spermidine, through synthetic oligopeptides, and finally to the protamines, histones, and synthetic polypeptides (Spitnik *et al.*, 1955; Felsenfeld and Huang, 1959, 1960; Matsuo and Tsuboi, 1966; Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Latt and Sober, 1967; Olins *et al.*, 1967, 1968, and references therein; Gabbay, 1968). The complexing phenomenon shows some specificity as to the nature and size of the components of the system (Felsenfeld and Huang, 1959; Szer, 1966a,b; Latt and Sober, 1967; Olins *et al.*, 1968), and the complexes are composed of stoichiometric ratios of nucleotide residue to cationic ligand which are characteristic for the system (Sober *et al.*, 1966; Tsuboi, 1967). Such complexes show greater thermal stability than the free polynucleotide components (Szer, 1966a,b; Tsuboi, 1967; Olins *et al.*, 1967, 1968). Finally, the solubility of the complexes has been found to be highly dependent upon experimental conditions (Spitnik *et al.*, 1955; Leng and Felsenfeld, 1966; Olins *et al.*, 1967; Tsuboi, 1967).

The above characterizations have been obtained largely by

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