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Circular Dichroism and Optical Rotatory Dispersion of α-Gliadin*

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ABSTRACT: The circular dichroism spectrum of α-gliadin, a wheat protein, was measured in 10^{-3} M HCl (pH 3), 10^{-5} M HCl (pH 5), and 10^{-5} M HCl plus 0.005 M KCl (pH 5). Bands at 222, 208, and *ca.* 191 mμ indicate the presence of some helical structure. The ellipticity 11,300 (deg cm²)/dmole^{-1} at 222 mμ for pH 5 solutions suggests about one-third helical structure. Although the protein aggregates when a small amount of salt is added at pH 5, no corresponding change was found in the circular dichroism associated with the peptide bonds. We infer that no major conformational change occurs when the protein aggregates. Bands associated with side-chain optical activity at 277 and 294 mμ became more intense when the protein aggregated. This change suggests that the environment of aromatic side chains changes in a specific way during interaction between protein subunits.

Optical rotatory dispersion and circular dichroism measured at various temperatures between 4 and 90° showed the molecule to be conformationally stable below about 30°. Above this temperature conformation changed progressively, the helical content decreasing, but even at 90° about 65% of the helical structure present at 25° remained intact.

Recently, Bernardin *et al.* (1967) separated α-gliadin from a mixture of wheat proteins by means of its specific aggregation properties. That is, increasing the salt concentration of aqueous solutions at pH 5 to only 0.005 M in KCl causes the protein to aggregate. The aggregated form can be collected by ultracentrifuging at about 133,000*g*. The particle weight of this aggregated α-gliadin must be in the millions. Electron micrographs of the aggregated protein show long fibrils with a uniform diameter of about 80 Å (Kasarda *et al.*, 1967). Similar fibrils may exist in solution. We have used circular dichroism and optical rotatory dispersion to study the conformation of dissolved α-gliadin and to look for changes in conformation upon aggregation.

For most experiments we used three solvent conditions: pH 3 (10^{-3} M HCl), pH 5 (10^{-5} M HCl), and pH 5 with KCl (10^{-5} M HCl–0.005 M KCl). At pH 3, α-gliadin dissolves readily, is insensitive to shearing forces, and is relatively insensitive to added salt; its apparent molecular weight is about 50,000 (Bernardin *et al.*, 1967). At pH 5 the protein dissolves slowly, is very sensitive to shearing forces (becomes turbid), aggregates at low salt concentrations, and precipitates or gels if the ionic strength is more than about 0.01. We have studied effects of temperature (4–90°) on solutions in these solvents and have also studied optical properties of solutions in 8 M urea, 90% D_2O, and 70% aqueous ethanol.

Materials and Methods[1]

The α-gliadin was prepared by the method of Ber-

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[1] Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

nardin *et al.* (1967), which yields samples of about 50 mg. These samples were not combined, so our measurements reflect lot variations as well as experimental error. A Radiometer pH meter (Model 22) was used to adjust the pH to within 0.1 pH unit of the reported values. Protein concentrations were determined by optical density measurements. We used a specific absorbance, $A^{1\%}_{1\text{ cm}}$, of 5.8 at 276 mμ and generally corrected for scattering by extrapolating the absorbance at 350–276 mμ, assuming a wavelength dependence of $1/\lambda^4$, and subtracting that value from the measured absorbance (Bernardin *et al.*, 1967). Occasionally, when the scattering was high, the correction was determined from a log–log plot of absorption against wavelength from 400 to 350 mμ extrapolated to 276 mμ. All absorption spectra were recorded on a Cary Model 15 spectrophotometer. Solutions were prepared by first dissolving the α-gliadin in 10^{-3} M HCl, filtering the solution through a 0.45-μ Millipore filter that had been washed to remove detergent (Cahn, 1967), and dialyzing the solution against the desired solvent. At pH 3, the solutions can be handled without difficulty, but at pH 5, particularly in the presence of added salt, they may become turbid when subject to even mild shear, for example, poured gently from one container to another. Therefore, we transferred pH 5 solutions by an infusion-withdrawal pump (Sage Model 249-1W). The flow rate through a 14-gauge Teflon syringe needle was kept less than 1 ml/min; a flow rate three times greater can cause some turbidity.

Circular dichroism measurements over the range 290–315 mμ were made with a Jasco ORD/CD/UV-5. This instrument had been calibrated by placing a standard solution of *d*-camphor in dioxane in the instrument and adjusting the voltage of the electrooptic modulator to give an instrument recording corresponding to a circular dichroic absorption ($\Delta\epsilon$) of 1.6 at 300 mμ, the value reported by Velluz *et al.* (1965). The circular dichroic absorption is

$$\Delta\epsilon = \epsilon_l - \epsilon_r = \frac{\Delta D}{cl} \qquad (1)$$

where ϵ_l and ϵ_r are the molecular absorption coefficients of left and right circularly polarized light, respectively, ΔD is the circular dichroic optical density (scale setting X reading on this instrument), c is the concentration in moles per liter, and l is the path length in centimeters. At least once in each 24 hr of instrument use, the calibration was checked by recording the 290-mμ maximum of a standard solution of *d*-10-camphorsulfonic acid (Eastman White Label) in water. Circular dichroism measurements were then corrected for small variations in instrument calibration. The circular dichroism data in this paper are reported in terms of mean residue ellipticity, $[\theta]$, in (deg cm²)/dmole, where

$$[\theta] = \frac{3300\Delta D}{cl} = \frac{3300\bar{M}_0\Delta D}{c'l'} \qquad (2)$$

and c' is the concentration of the optically active solute in grams per deciliter, l' is the path length in decimeters, and $\bar{M}_0$ is the mean residue weight, which we have taken as 117 for α-gliadin (Bernardin *et al.*, 1967). We have not applied the Lorentz correction for refractive index. Reduced mean residue ellipticity is close to 0.8 times the mean residue ellipticity for aqueous solutions (see Yang, 1968).

One set of circular dichroism measurements was made with a Cary 60 spectropolarimeter with circular dichroism attachment. This instrument, the Cary 6001, provides readings in ellipticity angle, $\theta°$, equal to $33.0 \times \Delta D$. The calibration was checked with a 1.00-mg/ml solution of *d*-10-camphorsulfonic acid in water at 25°. Cary Instruments provided the value 0.308° θ for this solution. We measured the circular dichroism of a similar solution with the Jasco instrument calibrated with *d*-camphor, and found that the ellipticity determined in this way was within 2% of the Cary value.

Optical rotatory dispersion measurements were made with a Cary 60 spectropolarimeter. This instrument was calibrated as described by Tomimatsu and Gaffield (1965). The optical rotatory dispersion data are reported as effective residue rotations, $[m']_\lambda$, where

$$[m']_\lambda = [\alpha]_\lambda\left(\frac{\bar{M}_0}{100}\right)\left[\frac{3}{(\eta_\lambda^2 + 2)}\right] \qquad (3)$$

and $[\alpha]_\lambda$ is the specific rotation, $\bar{M}_0$ is the mean residue weight, and η_λ is the refractive index of the medium. The specific rotation is equal to $100\alpha_\lambda/l'c'$, where α_λ is the observed rotation in degrees, l' is the path length in decimeters, and c' is the concentration of the optically active solute in grams per deciliter. Values for η_λ were estimated from the data of Foss and Schellman (1964). The Moffitt–Yang (1956) equation (eq 4) gave b_0 as the slope

$$[m'] = a_0\left(\frac{\lambda_0^2}{\lambda^2 - \lambda_0^2}\right) + b_0\left(\frac{\lambda_0^2}{\lambda_2 - \lambda_0^2}\right)^2 \qquad (4)$$

and a_0 as the intercept upon plotting $[m'](\lambda^2 - \lambda_0^2)/\lambda_0^2$ against $\lambda_0^2/(\lambda^2 - \lambda_0^2)$. A λ_0 of 212 mμ was assumed.

We used cells with path lengths of 1.00, 0.100, or 0.111 cm. Cell calibrations were determined by measuring the optical density of standard K_2CrO_4 solutions (Haupt, 1952) or the optical rotation of standard sucrose solutions (Yang and Samejima, 1963). Both the Jasco and Cary instrument used for optical rotatory dispersion measurements were fitted with cell holders of channeled aluminum blocks through which water was circulated to maintain cell and sample at any desired temperature between 0 and 90°. Water at a temperature controlled to ±1° was circulated by either a Haake Model KT-62 or Haake Model F bath. The sample temperature was checked with a Yellow Springs Instrument Co. 42-SC telethermometer. Measurements were made at 25° unless specified otherwise.

Results

The results are shown in Figures 1–6 and Tables I and II. All measurements were at least duplicated. Some measurements were repeated five times; the standard devia-

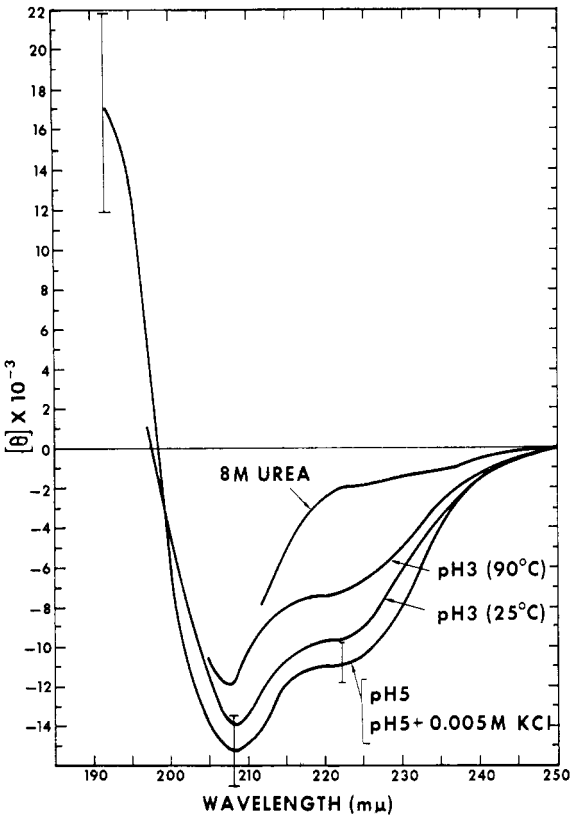

FIGURE 1: Circular dichroism of α-gliadin solutions. See text for detailed description of solutions. No significant difference was observed between the two pH 5 solutions. Measurements were made at 90° for one pH 3 solution; all other measurements were made at 25°.

TABLE I: Moffitt–Yang Parameters of α-Gliadin Solutions.

Solvent[a]	a_0	b_0
pH 3	−552	−110
pH 5	−506	−140
pH 5 + KCl	−491	−156
70% EtOH (pH 5)	−315	−150
90% D_2O + KCl	−492	−163
8 M urea	−830	+6

[a] See Materials and Methods for details.

TABLE II: Ellipticity at 222 and 294 mμ of α-Gliadin Solutions.

Solvent[a]	λ (mμ)	Av [θ]	Std Dev (5 samples)
pH 3	222	−9,680	1,300
pH 5	222	−11,000	900
pH 5 + KCl	222	−11,600	1,000
pH 5	294	−39	9
pH 5 + KCl	294	−65	5

[a] See Materials and Methods for details.

tions reported in Table II were derived from that number of measurements. Protein concentrations were generally determined on solutions removed from the cells after circular dichroism or optical rotatory dispersion measurements. Although solutions were clear during measurement of optical activity, subsequent transfers of solutions at pH 5 occasionally produced slight turbidity which increased the error of the ultraviolet absorption measurements. Accordingly, we estimate the error in our concentration determinations to have a 4% range at pH 5, and somewhat less at pH 3.

The circular dichroism measurements of α-gliadin below 250 mμ are shown in Figure 1 for aqueous solutions at pH 5.0 (10^{-5} M HCl), pH 5.0 with KCl (10^{-5} M HCl), pH 3.0 (10^{-3} M HCl), and with 8 M urea at pH 3.0. Also shown in Figure 1 is the circular dichroism spectrum of an α-gliadin solution at pH 3, measured at 90°. The solutions at pH 5, with and without KCl, gave identical circular dichroism spectra within our experimental error. The spectrum at this pH is characterized by two partly overlapping negative ellipticity bands with maxima at 222 and 208 mμ and a positive band with a maximum near 192 mμ. Crossover from negative to positive ellipticity occurs at 199 mμ. The 222-mμ band appears as a shoulder on the more intense 208-mμ band. Band positions are accurate to ±1 mμ except for the 192-mμ band, where high absorbance of the protein results in a poor signal-to-noise ratio. An estimate of the signal-to-noise ratio would not be meaningful in this region because the instrument gave a different tracing each time the spectrum was retraced. The vertical line at 192 mμ in Figure 1 is our estimate of the range of ellipticity derived from multiple tracings of two different samples. The vertical lines at 208 and 222 mμ in Figure 1 represent the standard deviations of measurements on five samples.

The circular dichroism spectrum of α-gliadin at pH 3 was quite similar to that at pH 5, but the two negative bands were slightly less intense and the crossover was displaced to 198 mμ.[2] The average intensity of the 222-mμ band is about 12% less (see Table II). Although this change appears not significant when compared with our standard deviations, we attempted to decrease our error by two sets of paired measurements of pH 3 and 5 solutions prepared from the same lot on the same day, recording the spectra on the same chart along with a base line. (No difference in base line was noted from 190 to 300 mμ for the pH 3 and pH 5 solvents.) Both sets of paired measurements showed decreasing ellipticity with decreasing pH of about the magnitude shown in Figure 1.

In 8 M urea at pH 3, α-gliadin showed weak negative ellipticity at 222 mμ; the ellipticity became more negative

[2] Although our crossover wavelength is accurate to only ±1 mμ, several comparisons show a 1-mμ shift to shorter wavelength for solutions at pH 3.

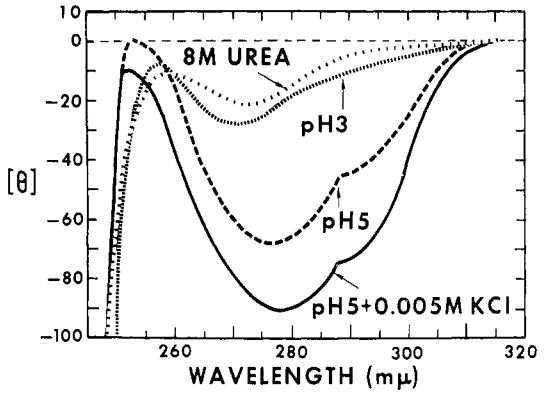

FIGURE 2: Circular dichroism of α-gliadin solutions.

at shorter wavelengths. Apparently there is a strong negative band with a maximum below this point. Strong absorbance of this solvent prevented measurements below 212 mμ. The effect of temperature was also observed. Intensities of the 222- and 208-mμ bands of the pH 3 solution were about 25% less at 90° than at 25°. This 65° change in temperature has roughly one-third of the effect of the added urea.

We observed relatively weak, but distinct, negative circular dichroism bands between 250 and 315 mμ at pH 5. These bands were intensified considerably when KCl was added, as shown in Figure 2. The major band had a maximum at 276–278 mμ with a distinct shoulder corresponding to a band at about 294 mμ. The ellipticity returned to zero at 253 mμ for the solution without KCl at pH 5 and nearly to zero for the solution with added KCl at pH 5. Additional fine structure may have been present but not resolved because of the weak circular dichroism but high optical absorbance of the protein in this range. The Cary and Jasco instruments showed about the same detail for these bands, which were measured in 1.00-cm cells usually using concentrations near 0.004 g/ml. Also shown in Figure 2 are the circular dichroism spectra of pH 3 solutions of α-gliadin with and without 8 M urea. The ellipticity of these solutions was extremely weak, but there was a residual negative band near 271 mμ and slightly more negative ellipticity near 250 mμ than observed at pH 5. The intensity of the band at pH 3 was increased only slightly by lowering the temperature to 4°. Adding 8 M urea also produced very little effect in this spectral region.

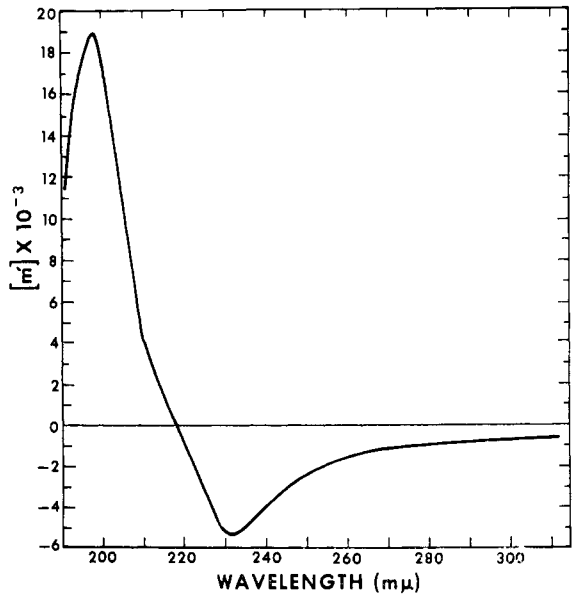

FIGURE 3: Optical rotatory dispersion of α-gliadin in 10^{-5} M HCl.

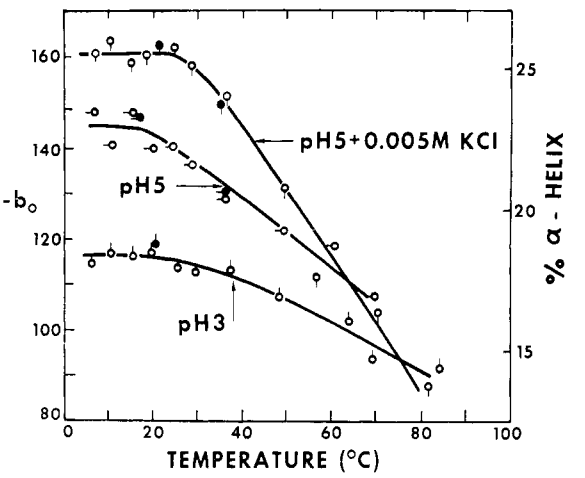

FIGURE 4: Moffitt–Yang parameter, b_0, as a function of temperature for α-gliadin solutions. Filled circles represent solutions reversed from the highest temperature reached.

The optical rotatory dispersion of α-gliadin at pH 5 is shown in Figure 3 from 190 to 310 mμ. The optical rotatory dispersion of the solutions at pH 5 with added KCl and at pH 3 was much like that at pH 5 without added KCl except for slight broadening of the 233-mμ trough at pH 3. All three spectra have minima at 233 mμ, crossovers at 217 mμ, and maxima at 198 mμ with shoulders at about 215 mμ. The effective residue rotation at 233 mμ was −5300 ± 5% for all three solutions.

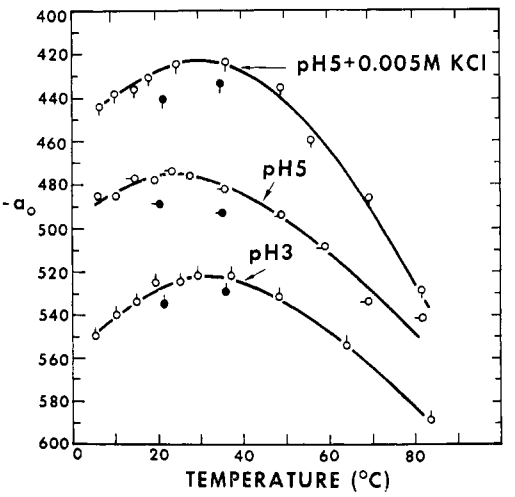

FIGURE 5: Moffitt–Yang parameter, a_0, as a function of temperature for α-gliadin solutions. Filled circles represent solutions reversed from the highest temperature reached.

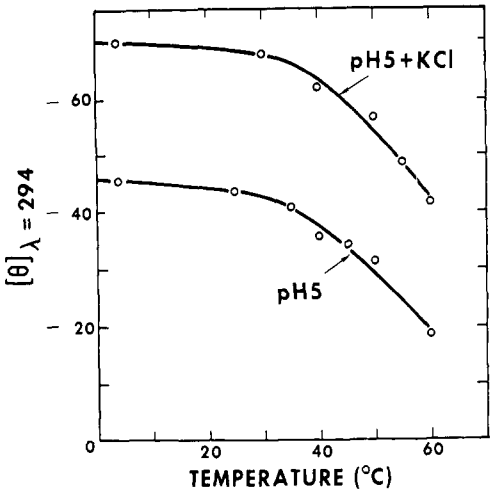

FIGURE 6: Ellipticity at 294 mμ as a function of temperature for α-gliadin solutions.

The a_0 and b_0 parameters of the Moffitt–Yang equation for α-gliadin in five different solvents are listed in Table I. There was a significant change in b_0 from −110 to −140 when the pH was changed from 3 to 5. This decrease was more readily observed than differences in the depth of the 233-mμ trough.

The a_0 and b_0 parameters of α-gliadin solutions at pH 3, 5, and 5 with KCl are plotted for the temperature range 5–85° in Figures 4 and 5. The data in these figures are from a single set of measurements. A duplicate set reproduced all of the general features of these figures but gave slightly different values for the parameters. The right-hand ordinate of Figure 4 provides a measure of α-helical content of the protein as a function of temperature based on the value of b_0 equal to −630 for 100% α-helix. Little change in helical content occurs below 20–30°; the apparent helical content decreases at higher temperatures. At pH 5 the decrease was more rapid than at pH 3; gliadin in all three solvents had similar values of b_0 at the higher temperatures. The pH 5 with KCl solution showed a more distinct break in the curve near 25° than the other solutions. This behavior is reproducible but its meaning is not known. The reversibility of thermal changes in b_0 was checked by returning to lower temperatures with the same solution after heating. The filled circles of Figure 4 show these measurements and indicate good reversibility after the α-gliadin solutions had been heated above 80°. We observed that pH 5 solutions occasionally became turbid above about 60°. This behavior was never encountered with pH 3 solutions. We are unable to account for it. Perhaps freshly prepared solutions are more stable than those a few days old. The behavior of a_0 with temperature (Figure 5) was unusual in that a_0 approached a maximum near 30° for all three solutions, then decreased at higher temperatures. The filled circles represent solutions heated to about 80° and cooled; the a_0 returned to within 3% of the original value. Thus, reversible changes in both a_0 and b_0 indicate reversibility in the decreasing helical content produced by heating above 30°.

The ellipticity, measured at 294 mμ, of solutions at pH 5 with or without KCl also changes with temperature as shown in Figure 6. Ellipticity changed very little below 30°, but decreased at higher temperatures. These data are from a single set of measurements on each of the two solutions. Duplicate measurements showed the same general features, but gave slightly different values and a slightly greater slope between 35 and 60°. Because of the small differences measured in a noisy region of the spectrum, we were satisfied to duplicate the slope in the range 4–30° and the break point near 30°.

Discussion

α-Helical Structure. No significant change in the optical activity associated with amide absorption bands was noted on adding KCl to solutions at pH 5. We infer that little or no conformational change occurs when the protein aggregates. We discuss later the possibility of small changes related to the side-chain circular dichroism bands. The negative circular dichroism bands at 222 and 208 mμ, crossover at 199 mμ, and apparent positive circular dichroism band at 192 mμ (Figure 1) for α-gliadin at pH 5 indicate that a substantial part of the polypeptide chain is right-handed α-helical (Holzwarth and Doty, 1965; Velluz and Legrand, 1965). The 233-mμ trough, peak at 192 mμ, and shoulder at 215 mμ in the optical rotatory dispersion are consistent with this inference. We shall assume that the properties of the α-helix reported in various studies of synthetic polypeptides are adequate to describe other helical structures in this protein as well.

The ellipticity (Table II) of the 222-mμ band of a pH 5 solution of α-gliadin is -11.0×10^3; that of the pH 5 with KCl solution is -11.6×10^3. If the ellipticity corresponding to 100% helix is -4.0×10^4 (Holzwarth and Doty, 1965; Cassim and Yang, 1967) and that corresponding to a random coil is $+3.0 \times 10^3$ (Beychok, 1966), then we calculate 33–34% helix in α-gliadin, for example $((-11.0\text{–}3.0) \times 10^3/(-40.0\text{–}3.0) \times 10^3 = 0.33)$. As Schellman and Schellman (1964) have pointed out, however, the "random structure" of a globular protein may be very different from the structure of a flexible polypeptide chain in solution as a consequence of increased interactions and restrictions occurring in the tightly folded globular form. For example, the tendency for charged side chains to occupy positions at the surface of the molecule and for nonpolar side chains to be buried in the interior restricts the arrangement of the polypeptide chain (Schellman and Schellman, 1964). Cassim and Yang (1967) have shown the existence of an isosbestic point near 205 mμ, where both the random coil and α-helical forms of poly-L-glutamic acid have the same ellipticity; they report an ellipticity of about -2.0×10^4 at this point. This isosbestic point could explain the appearance of circular dichroism spectra of certain proteins such as α-gliadin and lysozyme (Timasheff *et al.*, 1967b) where the 222-mμ band appears as a shoulder on the 208-mμ band. This observation may indicate that these proteins are simple mixtures of helix and random structure (for which the random coil is a reasonable model) and that (in these proteins and in this part of the spectrum) there is no substantial contribution to the

circular dichroism by β structure or side chains. Our ellipticity for α-gliadin of $-(1.2$ to $1.4) \times 10^4$ at 205 mμ is not in good agreement with this interpretation; however the presence of side-chain bands in this region of the spectrum could be responsible for the difference in ellipticities.

β Structure. Largely because the circular dichroism bands of the β structure in polypeptides are sensitive to environment (Iizuka and Yang, 1966; Timasheff *et al.*, 1967b), it is difficult to assign a value to the ellipticity of these bands corresponding to 100% β structure. If we assume the values reported by Townend *et al.* (1966) for these ellipticities, we then estimate that 20–40% β structure would have to be included in the synthesis to give a rough fit to our experimental curve near 199 mμ. Since infrared spectra (D. D. Kasarda and S. Kint, unpublished results) of α-gliadin films cast from H_2O solution (pH 5) show only weak shoulders near 1630 and 1685 cm^{-1}, it seems unlikely that α-gliadin has much β structure (Timasheff *et al.*, 1967a). The appearance of the spectrum between 1600 and 1700 cm^{-1} is similar to that of lysozyme which has about 10% β structure (Phillips, 1966), so that by visual comparison of these spectra we would make a crude estimate of about the same amount in α-gliadin. Consequently, we conclude that there is not enough β structure in α-gliadin to explain the appearance of the circular dichroism spectrum and that construction of the spectrum based on the circular dichroism spectra of synthetic polypeptides in α-helical, random coil, and β-structural forms tends to exaggerate β structure. A similar conclusion was reached by Greenfield *et al.* (1967) as a consequence of their attempt to synthesize the optical rotatory dispersion curves of proteins from those of synthetic polypeptides in the three forms. One reason for this discrepancy may be that ellipticity bands originating in aromatic side chains or disulfide bonds contribute significantly to the spectrum near 199 mμ. We do find some weak circular dichroism bands (Figure 2) attributable to side-chain optical activity in the 250–315-mμ region of the spectrum; these chromophores may have more intense circular dichroism bands at shorter wavelengths. These near-ultraviolet bands almost disappear at pH 3, however, without producing any change in the far-ultraviolet spectrum that cannot reasonably be attributed to a small decrease in helical content.

Neither β structure nor side-chain optical activity seems to provide an adequate explanation for the failure of a synthesis based on α-helical and random coil models to account for the circular dichroism spectrum of α-gliadin. We think it most likely that the problem lies with the assumption that a random polypeptide chain in solution is a good model for the non-ordered structure in the protein.

If we assume a value of -630 for the b_0 of the Moffitt–Yang equation to correspond to 100% α-helix, from our values of b_0 at pH 5 for α-gliadin (Table I) we calculate about 24% helix in the protein. This value is about 10% lower than that calculated from the 222-mμ circular dichroism band.

Since one of every seven residues in α-gliadin is proline (Bernardin *et al.*, 1967) which is unable to fit into an α-helix, we conclude either that proline in a compact folded protein does not place serious restrictions on the amount of helix formed by the polypeptide chain, or that the proline is not randomly distributed along the chain.

pH 3–5 Conformational Change. At pH 3, the 222- and 208-mμ circular dichroism bands of α-gliadin are slightly less intense than at pH 5. This decrease is consistent with a small conformational change in which the calculated helix content is decreased by about 5% as the acidity increases. This change in helix content agrees well with that calculated from the change in b_0 (Table I). Since this change occurs in the pH range in which carboxylate ions become protonated, it may result from unfolding a part of the polypeptide chain with two or more positively charged side chains. The decreases in ellipticity, however, could conceivably result from a loss in intensity of negative circular dichroism bands of side chains analogous to the decrease in intensity of the circular dichroism bands in the 250–315-mμ spectral region (Figure 2).

Urea Solutions. The ellipticity at 222 mμ is drastically reduced in 8 M urea solutions of α-gliadin (Figure 1), yet a small residual negative ellipticity remains. The ellipticity of polypeptides in the random coil conformation is slightly positive at 222 mμ (Holzwarth and Doty, 1965). The negative ellipticity we observe may indicate a stable portion of the protein molecule that resists unfolding in 8 M urea solutions and that contains some helical structure, or it may result from restrictions on the conformation of the unfolded chain. A third possibility is that the negative ellipticity is the result of residual optical activity of aromatic side chains of the unfolded polypeptide chain, as Hashizume *et al.* (1967) suggest for ribonuclease and other proteins. If the last possibility were correct, then we should subtract this residual activity from the activity of the native protein (pH 5) before estimating helix content. This would change our estimate of helical content in α-gliadin to 23–29%, depending on whether the ellipticity of the disordered molecule is near zero or $+3000$. As the wavelength decreased from 222 mμ, the ellipticity of 8 M urea solutions of our protein seemed to approach a fairly strong negative band below 200 mμ, which would correspond to the random coil conformation of the polypeptide chain (Holzwarth and Doty, 1965).

At pH 5, 70% ethanol or 90% D_2O has little effect on the conformation of the protein as measured by b_0 (Table I). Kretschmer (1957) obtained a helix content of about 35% from the b_0 of a crude gliadin mixture in 70% ethanol. This estimate is somewhat higher than our result from b_0. Beckwith and Heiner (1966) reported a b_0 of -116 for an α-gliadin preparation in 0.3 M acetic acid. Their value compares quite well with ours of -110 in dilute HCl. Cluskey and Wu (1966) have reported values of b_0 of -96 for a gliadin mixture in 0.01 M acetic acid and -120 for a gliadin mixture in aluminum lactate at pH 3.2. These values are close to our values at pH 3. We conclude that the many components of a crude gliadin mixture (Woychik *et al.*, 1961) have conformations quite similar to those of the two or possibly three recognized components (Bernardin *et al.*, 1967) of our α-gliadin preparation.

Side-Chain Optical Activity. In the 250–315-mμ region of the spectrum, we observed some weak negative circular dichroism bands that were poorly separated from one another, but distinctly resolved from the circular dichroism bands of the peptide linkages (Figure 2); they evidently result from optically active transitions of aromatic side chains or disulfide bonds (Beychok, 1966; Beychok and Breslow, 1968). Although only residual optical activity was observed for pH 3 solutions of α-gliadin, upon raising the pH to 5 we found that at least two negative bands appeared in the spectrum, the more intense band at 277 mμ with a shoulder corresponding to a band at about 294 mμ. In addition, the ellipticity at 252 mμ, though never greater than zero, became relatively more positive, so that there may be a weak positive band in this region. The changes in ellipticity near 252 mμ might result, however, from changes in the shapes and intensities of negative circular dichroism bands adjoining this region. Other circular dichroism bands than those mentioned may be present, but unresolved.

The appearance of 277- and 294-mμ bands upon raising the pH from 3 to 5 most likely results from a conformational change which restricts the motion of the side-chain chromophores. The conformational change may be the same one that brings about increased intensity of the 222- and 208-mμ circular dichroism bands (Figure 1) and a change in b_0 (Table I) in going from pH 3 to 5. Alternatively the optical activity at 277 and 294 mμ could result from increased asymmetry of the environment of already restricted side chains upon creation of nearby negatively charged carboxyl groups. Beychok and Breslow (1968) have studied the circular dichroism of oxytocin and found that the state of charge of the α-amino group affected the circular dichroism near 280 mμ. The 277- and 294-mμ bands of α-gliadin show a significant increase in intensity when pH 5 solutions are made only 5 mM in KCl (Figure 2). This subtle change in environment brings about aggregation of the protein (Bernardin *et al.*, 1967; Kasarda *et al.*, 1967) and the intensification of these near-ultraviolet circular dichroism bands may result from a direct involvement of aromatic side chains in the interaction between protein subunits, or the effect of an accompanying conformational change on these side chains or on disulfide bonds.

We can only speculate on the possible origins of the near-ultraviolet circular dichroism bands of α-gliadin. It is difficult to assign exact band positions from our measurements because the bands overlap. In addition, a circular dichroism band need not occur at the same wavelength as its absorption band for a forbidden transition, but may be shifted as much as 10 mμ to the red according to Moffitt and Moscowitz (1959). Beychok *et al.* (1966) found negative circular dichroism bands at 272 and 296 mμ in carbonic anhydrase B, a protein without disulfide, and show evidence that the 272-mμ band results from tyrosine. The 277-mμ band of α-gliadin may be analogous to their 272-mμ band and may also result from tyrosine. The 294-mμ band of α-gliadin might be caused by a tryptophan transition but we cannot rule out a tyrosine transition. Also, Beychok and Breslow (1968) have reported circular dichroism bands near 280 mμ in peptides that have only a disulfide bond and no aromatic side chains.

Analysis of our circular dichroism results is based on an average residue weight of 117 for α-gliadin rather than on moles of protein. The molecular weight of the fundamental subunit of α-gliadin has not yet been determined, but may be near 22,000 (Bernardin *et al.*, 1967) and on this basis the protein has 6 residues of tyrosine, 2 residues of tryptophan, and 2 disulfide bonds per 191 amino acid residues. If the ellipticities of the near-ultraviolet circular dichroism bands result from these side chains, we should multiply the measured ellipticities by some factor in order to provide a more direct relationship to the optically active chromophores. For example, if the 277-mμ bands results from tyrosine alone, and if all the tyrosine residues are optically active, we should multiply the observed ellipticity at this wavelength by about 30 to give an ellipticity of −2700 referred specifically to tyrosine. If not all tyrosine residues are active, the factor should be even higher. Ellipticity of −2700 is greater than has been reported for the free amino acid (+1320 at 274 mμ according to Beychok, 1966) and of opposite sign; the enhanced activity of the chromophore and the different sign of the circular dichroism upon incorporation of the chromophore into the protein must result from a different and more asymmetric environment.

Temperature Dependence of the Conformation. The Moffitt–Yang parameter, b_0, shows no dependence on temperature up to about 25° for pH 5 with KCl solutions of α-gliadin (Figure 4). A break in the plot of b_0 *vs.* temperature occurs near 25°; beyond this point b_0 falls off quite rapidly with increasing temperature. Evidently the conformation of the protein is stable up to the "break" or transition point with the polypeptide chain progressively unfolding beyond it. Somewhat similar behavior was observed for pH 5 and 3 solutions, but the break in the curve near 25° was less evident and the change in b_0 was more gradual so that the protein seemed to approach the same state in all three solutions at higher temperatures. Apparently the structure gained in raising the pH from 3 to 5 is lost on raising the temperature of pH 5 solutions above about 60°. The conformational change brought about by ionizing some of the carboxyl groups can be reversed by raising the temperature. The protein seems quite stable conformationally in that raising the temperature of a pH 3 solution to 90° results in only about a 6% decrease in helix content as measured by the decrease in the ellipticity of the 222-mμ band (Figure 1). The effects of high temperature seem reversible at least for the relatively short exposures necessary for our measurements. α-Gliadin is notable for its large number of amide side chains; about one of every three residues is glutamine (Bernardin *et al.*, 1967). Perhaps hydrogen bonding of these amide side chains contributes to the stability of helices in the protein, particularly if these lengths of helix are buried in the interior of the protein molecule and not exposed to the solvent, which would compete for bonding sites. α-Gliadin has many hydrophobic side chains and very few side chains that can assume a charge (Bernardin *et al.*, 1967), so it is likely that some helical segments are buried in

hydrophobic regions. Hydrogen bonding of glutamate (carboxyl) side chains has been suggested as a source of α-helix stabilization in poly-L-glutamic acid (see Fasman (1967) for discussion and references).

The change in a_0 with temperature (Figure 5) is unusual in that a_0 becomes less negative with increasing temperature up to 25–35°, where a maximum occurs, then decreases at still higher temperatures. The maxima in Figure 5 correspond closely to the transition temperatures in the b_0 plots of Figure 4. Because a_0 generally becomes less negative when the environment becomes less polar (Carver *et al.*, 1966), the observed change may indicate protein aggregation with resulting decrease in exposure of the peptide chains to the solvent (Herskovits *et al.*, 1964). Although possible, this explanation seems unlikely to apply to all three solutions equally in the low temperature range. Consequently, we cannot offer a consistent explanation for this behavior. The increasingly negative values of a_0 at temperatures above 35° most likely indicate polypeptide chain unfolding.

The near-ultraviolet circular dichroism bands show little change with temperature up to about 30° (Figure 6). Beyond 30° the ellipticity of both pH 5 solutions falls off with increasing temperature. This could result from increased mobility of aromatic side chains attached to a part of the polypeptide chain that unfolds with increasing temperature. These aromatic side chains might also be attached to the same part of the chain involved in the pH 3–5 conformational transition since distinct near-ultraviolet bands are not evident at pH 3, but only at pH 5 (Figure 2).

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