OPTICAL ROTATORY DISPERSION OF WHEAT GLUTEN, GLIADIN, AND GLUTENIN IN ACETIC ACID AND ALUMINUM LACTATE SYSTEMS[1]

J. E. CLUSKEY AND Y. V. WU

ABSTRACT

The conformations of gluten, gliadin, and glutenin were studied by optical rotatory dispersion in $0.01N$ acetic acid and also in $0.008M$ aluminum lactate-lactic acid buffer, pH 3.1, $\mu = 0.05$, with and without $3M$ urea. The wave-length range covered was from 600 to around 220 mμ or lower. All three proteins in each solvent exhibited a negative Cotton effect at 233 mμ (specific rotation ranging from $-3,900°$ to $-6,300°$). Values of b_0 from Moffitt-Yang plots were interpreted by the conventional method ($b_0 = -630$ for 100% right-handed alpha-helix). The optical rotatory dispersion data were also treated by a modified two-term Drude equation of Shechter and Blout. Results indicate that gliadin contains more alpha-helix than glutenin and whole gluten. The amount of alpha-helix in a given protein is the same in acetic acid and aluminum lactate. With inclusion of $3M$ urea in the aluminum lactate buffer, the helix content decreases slightly for each protein. Glutenin and gliadin in acetic acid and in aluminum lactate probably are mixtures of random coil and helix. These results supplement those reported earlier by Wu and Cluskey using different solvent systems.

Wheat gluten, the major protein complex of wheat flour, has been investigated most actively in the past 10 years. Since Osborne's (1) classic work in 1907, in which he fractionated gluten into gliadin and glutenin by 70% ethanol precipitation, new fractionation methods have appeared as new research tools and analytical techniques. Electrophoresis, both moving-boundary and starch-gel with an aluminum lactate buffer (2), demonstrated heterogeneity of proteins in wheat gluten. Amino acid composition (3) and hydrogen-ion equilibrium (4,5) studies of gluten, gliadin, and glutenin have appeared recently. Within the past 3 or 4 years, improved instrumentation for optical rotation measurements has enabled chemists to extend the region of measurement from the near-ultraviolet to the far-ultraviolet (185 mμ). This technique of optical rotatory dispersion has been applied at this Laboratory to investigate conformation of the gluten proteins.

Previous dispersion studies, in which the wheat gluten proteins were measured in formic acid and 70% ethanol (6) and also in urea and hydrochloric acid solutions (7), have now been extended. Three

solvent systems, aluminum lactate-lactic acid, with and without $3M$ urea, and acetic acid, commonly employed in wheat gluten research, were used in the work reported here.

Materials and Methods

The gluten, gliadin, and glutenin, from Ponca hard red winter wheat, were prepared according to the procedure of Jones et al. (8). These same preparations were used in hydrogen-ion equilibria (4,5) and the conformational (9) studies of Wu and Dimler. The concentration of each protein solution was determined at 276 mμ in a Cary-14 recording spectrophotometer (Applied Physics Corporation). The same conversion factors employed by Wu and Dimler (4,5) were used. Reagent grade chemicals and deionized distilled water were used.

Measurements of optical rotatory dispersion were made at 27°C. with a Cary-60 recording spectropolarimeter. This instrument was calibrated with sucrose solutions (National Bureau of Standards sucrose), which gave data that fitted a one-term Drude equation from 600 to 250 mμ. The rotation of the sucrose solution was the same with or without a cell containing a K_2CrO_4 or formamide solution of optical density 1.2–1.4 in series with the sample cell. The details of the calibration method and the results obtained were essentially the same as those given by Yang and Samejima (10). The spectropolarimeter was also calibrated by a quartz control plate certified by the National Bureau of Standards. The spectropolarimeter reading agreed with that given by the control plate within experimental error. When the slit opening was too wide, a false, higher reading resulted. The dispersion of the spectropolarimeter prisms is quite large at long wave lengths and decreases rapidly as the wave length decreases. A constant band pass of 1 mμ was used in the experiments to allow the slit opening to be small at long wave lengths and to eliminate any possible error from dispersion of the prisms.

For the most part, 1-cm. cells were used; for glutenin measurements between 600 and 300 mμ, however, 2.5- and 10-cm. cells were needed. The concentrations of the proteins ranged from 1 to 0.002%. A solvent blank was subtracted from the observed rotation of the protein solution. The optical rotation was measured from 600 to 200 mμ for proteins in acetic acid and from 600 to 225 mμ in aluminum lactate solutions. While the gluten and gliadin dissolved completely, the glutenin did not and had to be clarified by centrifugation. Approximately 70% of the glutenin was recovered.

The pH's were measured with a Radiometer pH meter 4 cali-

brated with standard phosphate and phthalate buffers prepared as described by Bates (11).

Results and Discussion

Gluten, gliadin, and glutenin all give negative Cotton effects and show a minimum at or near 233 mμ with the three solvent systems used. Figure 1 shows the Cotton effect of gluten in 0.01N acetic acid.
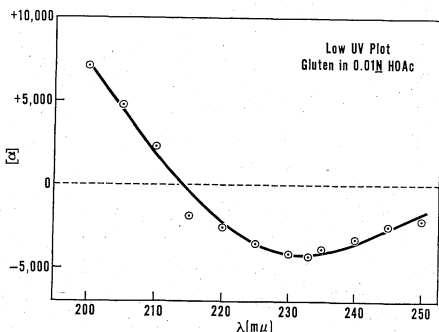

Fig. 1. Cotton effect of gluten in 0.01N HOAc, pH 3.44. Gluten concentration 0.0182%, 1-cm. cell.

A large negative Cotton effect at 233 mμ, shown by poly-γ-benzyl-L-glutamate, has been associated with the right-handed helix, whereas a large positive Cotton effect, as exhibited by poly-β-benzyl-L-aspartate, has been correlated with a left-handed helix (12). Simmons and co-workers (12) suggest that the reduced residue rotation, [R′] (residue rotation corrected for refractive index of the solvent), at 233 mμ may be used as an approximate measure of alpha-helix content and assumed a mean value of $[R']_{233} = -12,700$ or 100% helix, $-1,800$ for 0% helix, and a linear interpolation. $[R']_\lambda = 3 \, [a]_\lambda \, M/(n^2 + 2)100$, where M is average residues weight, n is refractive index of solvent, and $[a]_\lambda$ is the specific rotation defined in the usual way. Yang and McCabe (13) have recently reported $[R']_{233}$ values of $-13,700$ and around $-16,600$ for different samples of poly-L-glutamic acid.

Optical rotatory results were also analyzed by the method of Moffitt (14):

$$[a]_\lambda = \frac{(100)}{(M)} \frac{(n^2 + 2)}{3} \left[\frac{a_0 \, \lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0 \, \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \right]$$

where b_0 is the term that characterizes the helical form of the polypeptide and a_0 is a simple dispersion term made up of both helix and residue contributions. The value of λ_0 was taken to be 212 mμ in accordance

with that found best for poly-γ-benzyl-L-glutamate in a variety of solvents (15). The parameter b_0 was calculated from $\left[(M/100) \left[3/(n^2 + 2) \right] \right] /\lambda_0^4$ times the slope of a plot as shown in Fig. 2. Figure 2
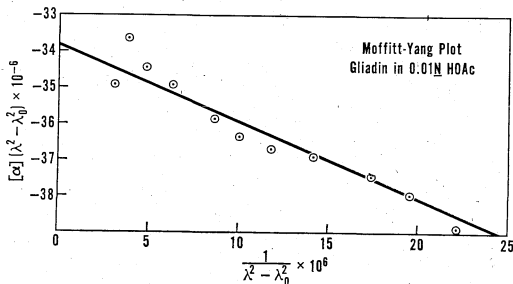

Fig. 2. Moffitt plot for gliadin in 0.01N HOAc, pH 3.67. Gliadin concentration 0.911%, 1-cm. cell.

shows a Moffitt plot of the dispersion data for gliadin in 0.01N acetic acid. The percent helix calculated by the Moffitt equation deserves further explanation.

It is known that both polypeptides and proteins with low helical content may show simple dispersion. Since the b_0 values calculated from the Moffitt equation were small, a one-term Drude plot was feasible in treating the rotatory data (16). The Drude equation may be expressed:

$$\lambda^2 \, [a] = \lambda_c^2 \, [a] + K$$

Figure 3 shows a one-term Drude plot for gluten in 0.01N HOAc. The parameter λ_c is derived from the square root of the slope of the straight line and K from the intercept.
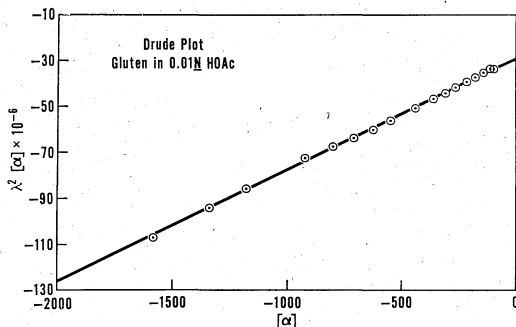

Fig. 3. One-term Drude plot for gluten in 0.01N HOAc, pH 4.06. Gluten concentration 0.941%, 1-cm. cell.

In 1964 Shechter and Blout (17) reported a novel analysis of visible and near-ultraviolet rotatory dispersion data of aqueous solutions of polypeptides and proteins having alpha-helical or random conformations, or mixtures of both. Their expression was designated as a modified two-term Drude equation:

$$[R'] = \frac{A_{(a,\rho)\,(193)}\,\lambda_{193}^2}{\lambda^2 - \lambda_{193}^2} + \frac{A_{(a,\rho)225}\,\lambda_{225}^2}{\lambda^2 - \lambda_{225}^2}$$

where the subscripts of the λ's are the wave lengths in millimicrons. These wave lengths relate to cross-over points. Two subscripts are used with each A constant. The first subscript in parentheses denotes the conformations to which A is related; a is used for the alpha-helix, ρ is used for a random conformation. The second subscript of A is the wave length of the cross-over as mentioned above. For molecules that consist only of random and alpha-helical segments, Cotton effects at 225, 198, and 193 mμ are important. The two-term equation can be written:

$$[R']\frac{\lambda^2 - \lambda_{193}^2}{\lambda_{193}^2} = A_{(a,\rho)\,(193)} + A_{(a,\rho)\,225}\frac{\lambda_{225}^2}{\lambda_{193}^2} + A_{(a,\rho)\,225}\cdot\frac{\lambda_{225}^2 - \lambda_{193}^2}{\lambda_{193}^2}\frac{\lambda_{225}^2}{\lambda^2 - \lambda_{225}^2}$$

When $[R']\,(\lambda^2 - \lambda_{193}^2)/\lambda_{193}^2$ *vs.* $\lambda_{225}^2/(\lambda^2 - \lambda_{225}^2)$ is plotted, one should get a straight line where $A_{(a,\rho)\,225}\,[(\lambda_{225}^2 - \lambda_{193}^2)/\lambda_{193}^2]$ is the slope and $A_{(a,\rho)\,(193)} + A_{(a,\rho)\,225}\,(\lambda_{225}^2/\lambda_{193}^2)$ is the intercept. Figure 4 illustrates the Shechter-Blout plot of gluten in 0.01M HOAc. This modified two-term Drude plot is valuable in cases where rotation measurements in the
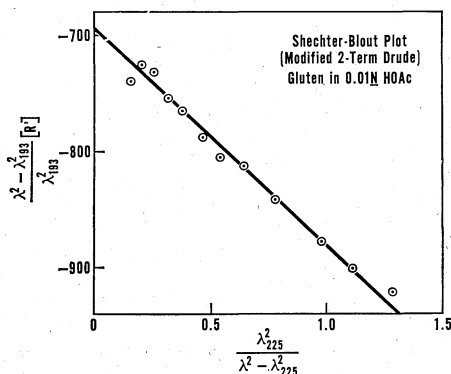

Fig. 4. Modified two-term Drude plot (Shechter and Blout) for gluten in 0.01N HOAc, pH 4.06. Gluten concentration 0.941%, 1-cm. cell.

far-ultraviolet are not possible because of solvent or solute absorption.

Equations for percent helix, H, were also developed by Shechter and Blout (17) and are as follows:

$$H_{193} = \frac{A_{(a,\rho)(193)} + 750}{36.5}$$

$$H_{225} = -\frac{A_{(a,\rho)225} + 60}{19.9}$$

H_{193} agrees with H_{225} for polypeptides and proteins in alpha-helical or random conformations, or mixtures of the two in aqueous solution. When H_{193} and H_{225} do not agree, the presence of other structures is indicated. The precision of this method is estimated to be better than $\pm 5\%$ helix content, the variation being due to experimental error in measurement of rotation and concentration.

TABLE I

OPTICAL ROTATORY PROPERTIES OF WHEAT GLUTEN, GLIADIN, AND GLUTENIN IN ACETIC ACID AND ALUMINUM LACTATE BUFFER SOLUTIONS AT 27°C.

SOLVENT	$[a]_{589}$	λ_c	a_0	b_0	HELIX CALCULATED [a]			
					(1)	(2)	(3)	Av.
	degrees	$m\mu$	degrees	degrees	%	%	%	
Gluten								
1) 0.01N HOAc pH 3.5–4.1	− 98	219	−547	− 89	14	18	14	15
2) 0.008M AlLac-HLac pH 3.2–3.3, $\mu = 0.05$ [b]	− 97	222	−560	− 74	12	22	20	18
3) 2) above + 3M urea pH 3.1–3.2	−114	220	−687	− 52	8	c	17	12
Gliadin								
1) 0.01N HOAc pH 3.4–3.8	−114	225	−694	− 96	15	31	24	23
2) 0.008M AlLac-HLac pH 3.2, $\mu = 0.05$ [b]	−112	231	−647	−120	19	24	26	23
3) 2) above + 3M urea pH 3.1–3.2	−122	226	−748	− 97	15	24	24	21
Glutenin								
1) 0.1M HOAc pH 3.4–4.1	−102	220	−592	− 59	9	16	16	14
2) 0.008M AlLac-HLac pH 3.2, $\mu = 0.05$ [b]	− 99	221	−574	− 63	10	13	20	14
3) 2) above + 3M urea pH 3.2	−126	213	−726	− 31	5	17	15	12

[a] Column (1), by Moffitt equation with $\lambda_0 = 212$ mμ, $b_0^{100} = -630$ and $b_0^{0} = 0$.

    Column (2), average value with $[R']_{233} = -12,700$ for 100% helix, and $-1,800$ for 0% helix, and $[R']_{233} = -16,600$ for 100% helix and $-1,800$ for 0% helix.

    Column (3), average of H_{193} and H_{225} values from modified two-term Drude equation.

[b] Nominally 0.05. See ref. 8.

[c] $[a]$ is not reproducible when concentration is changed; however, a Cotton effect does occur here at 233 mμ.

A summary of the results of this study appears in Table I. The $[a]_{589}$ and λ_c values were obtained from the Drude equation and are consistent with values for proteins of low helical content. The a_0 and b_0 parameters are from the Moffitt equation (14) with $\lambda_0 = 212$ mμ. In a previous paper, Wu and Cluskey (7) showed that when $\lambda_0 = 216$ mμ for gluten in 0.002N HCl, no improvement in linearity in the plot was evident. The percent helix calculated from the Moffitt equation is given in column (1). Column (2) is an average of calculation of percent helix according to the proposals of Simmons and colleagues (12) and of Yang and McCabe (13). The two calculations differ only in the values assigned to $[R']_{233}$ for 100% helix. Column (3) gives the estimate of helix content as calculated by the modified two-term Drude equation of Shechter and Blout (17). Since the values of H_{193} and H_{225} were quite close, the average value is given.

In principle, the values for five parameters of gluten, $[a]_{589}$, λ_c, a_0, b_0, and percent helix should be the sum of those of the weight fractions of gliadin and glutenin in the same solvent. Assuming the gliadin and glutenin to be in 50/50 ratio, this summing generally holds true. The a_0 and b_0 values of gluten in the three solvents compared in this way with gliadin and glutenin may reflect some interactions between the gliadin and glutenin in the whole gluten. Interaction would be most indicated in the a_0 values in the aluminum lactate buffer. Table I shows that each protein has practically the same optical rotatory properties in solvents 1 and 2, although a small difference is noted in the percent helix of wheat gluten. When urea is incorporated into solvent 2 an increase in $-[a]_{589}$ and $-a_0$ and a decrease in λ_c and $-b_0$ were effected. The decrease in b_0 is reflected in (1) in the percent helix tabulation.

In 1957, Kretschmer (6) calculated the alpha-helical content of gluten and gliadin by the Moffitt equation using $b_0^{100} = -640°$. Helical contents of about 35% were calculated for gliadin in 70% ethanol and for gluten in dilute formic acid. These percentages are higher than those reported in column (1) in Table I and no doubt are the result of differences in solvent.

That gluten proteins contain large amounts of proline is well known. Wu and Dimler (5) have reported 13.4% proline for glutenin, 17.6% for gliadin, and 16.2% for gluten. The proline residue with its pyrollidine ring does not fit well into a right-handed alpha-helix. Low alpha-helix content is consistent with high proline content.

We show that: 1) gluten, gliadin, and glutenin all have fairly low helix contents of the order of 20% in acetic acid and aluminum lactate buffers; 2) gliadin has more helix content than glutenin in all systems;

3) $0.01M$ acetic acid and aluminum lactate buffer $\mu = 0.05$ are almost identical as solvents for optical rotatory measurements; 4) the helix content, based on the residue rotation at 233 mμ, is subject to more error because the high absorption of protein solution necessitates the use of a dilute solution at that wave-length range and because there are some questions on the proper residue rotation values for 100 and 0% helix; 5) when aluminum lactate buffer contains $3M$ urea, the average calculated helix content of the proteins shows a small decrease; 6) H_{193} and H_{225} values for gluten, gliadin, and glutenin agree quite well in all the solvents and indicate they probably are mixtures of random coil and helix.

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