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Optical Rotatory Dispersion, Circular Dichroism, and Infrared Studies of Wheat Gluten Proteins in Various Solvents[1]

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ABSTRACT

The influence of various solvents upon the conformation of gluten proteins, glutenin and gliadin, has been studied by optical rotatory dispersion (ORD), circular dichroism (CD), and infrared (IR) absorption measurements. From ORD data an alpha-helix content of 38% has been calculated for gliadin in trifluoroethanol; 35% for glutenin. Conversely, little or no alpha-helix was found when the proteins were dispersed in 8M urea solutions. CD measurements made on gliadin and glutenin showed the presence of alpha-helical structure. IR absorption spectra of gliadin indicated the presence of alpha-helical and unordered conformations.

Conformation of wheat gluten proteins in 3M urea, hydrochloric and acetic acids, and aluminum lactate systems from optical rotatory disperson (ORD) data has already been reported (1,2,3). In terms of Moffitt-Yang, Cotton effect, and Shechter-Blout calculations, both gliadin and glutenin are likely a mixture of alpha-helix and unordered structures; however, gliadin contains more alpha-helix than glutenin. Further, with an increase in ionic strength, there is an increase in helical content for glutenin but no significant change for gliadin. ORD studies of gluten, glutenin, and gliadin showed that the amount of alpha-helix in each protein is solvent dependent.

Since a comprehensive study of the conformation of a protein in solution requires a variety of solvents including helix- and random structure-forming ones and, furthermore, since infrared (IR) and circular dichroism (CD) data are desirable in addition to ORD in such a study, ORD, IR, and CD results of gliadin and glutenin in various solvent systems are detailed here.

MATERIALS AND METHODS

Glutenin and gliadin from Ponca hard red winter wheat were prepared according to the procedure of Jones et al. (4). The concentration of each protein solution was determined in a Cary 14 recording spectrophotometer (Cary Instruments) at 276 nm. The same conversion factors were employed as reported in previous work (1). Reagent-grade chemicals were used whenever available.

ORD was measured at 25°C. with a Cary 60 recording spectropolarimeter. The optical rotation was measured from 600 to 220 nm. in general and to 188 nm. in the far ultraviolet studies. Calibration of this instrument and procedures followed are described elsewhere (1).

Measurements of CD between 300 and 188 nm. were carried out at 26.5°C. in a Cary Model 6001 CD accessory for the Cary 60 instrument.

Optical rotatory data were analyzed by the Moffitt equation (5) and the modified two-term Drude equation of Shechter and Blout (6,7) in aqueous and organic solvents. Details of data treatment with these equations were reported earlier (2).

IR absorption was measured with a Perkin-Elmer 621 grating IR spectrophotometer. Sodium chloride and potassium bromide cells, 0.025 and 0.1 mm. path lengths, respectively, were used for the proteins in the organic solvents, whereas 0.1 mm. path length calcium fluoride cells were used in the D_2O-DCl work. Concentrations were maintained chiefly at 1 to 1.5% protein. The IR measurements were made on the absorbance scale with an expanded wave length setting between 1,700 and 1,450 cm.$^{-1}$ (frequency). The spectrophotometer was calibrated with water vapor.

RESULTS AND DISCUSSION

Solvent greatly affects the alpha-helix content of wheat glutenin and gliadin (Table I). Both a_0 and b_0 parameters of the Moffitt-Yang plots are given. The a_0 parameter represents intrinsic residue rotations, which are present irrespective of alpha-helix, and those due to interactions within the alpha-helix. Therefore, a_0

TABLE I. ROTATORY DISPERSION PROPERTIES OF WHEAT GLUTENIN
AND GLIADIN IN VARIOUS SOLVENTS

Wheat Protein	Solvent	a_0 (deg)	b_0 (deg)	Percentage α-Helix Calculated[a]		
				(A)	(B)	Average
Glutenin	F_3CCH_2OH	−338	−235	29	41	35
	2-$ClCH_2CH_2OH$[b]	−213	−199	32	37	34
	8M Urea, pH 6.3	−765	−5	1	9	5
	$(CF_3)_2CO \cdot 1\frac{1}{2} H_2O$	−919	−12	2	10	6
Gliadin	F_3CCH_2OH	−482	−263	32	45	38
	96% 2-$ClCH_2CH_2OH$[b]	−362	−264	41	45	43
	8M Urea, pH 5.6	−847	0	0	12	6
	$(CF_3)_2CO \cdot 1\frac{1}{2} H_2O$	−1036	−11	1	8	4

[a](A) Percentage helix calculated from the Moffitt equation with $b_0 = -816$ in F_3CCH_2OH; $b_0 = -636$ in 2-chloroethanol; in others, $-b_0 = 1701-730.3$ n for 100% alpha-helix (8) where n is the refractive index. $\lambda_0 = 212$ nm., $b_0 = 0$ for 0% alpha-helix in all cases. (B) $H = (A_{193} - A_{225} + 650)/55.8$ from modified two-term Drude equation by Shechter-Blout (6,7) and is independent of solvent.

[b]N. W. Taylor and H. F. Zobel, unpublished data.

should be solvent dependent (9). In aqueous solution the b_o is a function of the alpha-helical backbone, independent of side chain and environment. The percentage alpha-helix was calculated not only from the Moffitt equation (Table I, A) but also by the modified two-term Drude equation (Table I, B) of Shechter and Blout (6,7). The two alpha-helix estimations, A and B, are in closer agreement as the alpha-helix content increases. An increase in $-b_o$ increases percentage alpha-helix. Glutenin and gliadin have fairly low alpha-helix contents of the order of 20% in acetic acid and aluminum lactate buffers—common gluten protein dispersants (2). Table I illustrates how the alpha-helicity can be altered in either direction by changing the solvent system. For both proteins, the 8M urea solvent is very effective in disrupting alpha-helices and, on the one hand, can be said to make the molecule almost completely unordered. On the other hand, glutenin and gliadin can be said to contain maximum alpha-helix with trifluoroethanol and 2-chloroethanol. As previously demonstrated (2), gliadin has relatively more alpha-helix than glutenin. Two fluorinated solvents—trifluoroethanol and hexafluoroacetone sesquihydrate—were used as glutenin and gliadin dispersants. The first is an alpha-helix former; the second, an unordered structure-forming solvent (10). The alpha-helix content was calculated by the methods of Moffitt-Yang and Shechter-Blout. Averages of these two calculations showed that glutenin has 35% alpha-helix and gliadin 38% alpha-helix in trifluoroethanol. When glutenin in trifluoroethanol was dialyzed against 0.01N acetic acid, the amount of alpha-helix became 14%, which is the same as glutenin in 0.01N acetic acid (2). Since the ORD parameters of glutenin are unchanged in 0.01N acetic acid even when trifluoroethanol is used to dissolve the glutenin initially, trifluoroethanol can be used to dissolve glutenin, and then studies can be made in other desired solvents by dialysis. The alpha-helix content of glutenin and gliadin in hexafluoroacetone sesquihydrate is reduced to 4 to 6% although the Cotton effect at 233 nm., characteristic of alpha-helix, is still present.

The far ultraviolet ORD of gliadin and glutenin in various solvents was measured from 250 to 188 nm. The ORD measurements of gliadin in 0.001N HCl at pH 3 are plotted in Fig. 1. The curve shows the alpha-helical minimum at 233 nm. with reduced mean residue rotation $[m']$ of $-4,300°$, and a maximum at 195 nm. with $[m']$ of 18,100°. A synthetic mixture of 25% alpha-helix and 75% random structure from Yang's (11) alpha-helical and random structure data for poly-L-glutamic acid gives a maximum $[m']$ at 194 nm. The 195 nm. maximum for gliadin agrees with this calculation. The reduced mean residue rotation at 233 nm. compares relatively closely with the value for alpha-gliadin reported by Kasarda et al. (12). In the same solvent, glutenin gave an $[m']$ of $-4,200°$. Values of $[m']_{233}$ and $[m']_{198}$ are listed in Table II. It is also possible to generate a theoretical curve for protein based on amounts of alpha-helix, beta-structure, and unordered conformation. However, such synthetic curves do not always correspond with the experimental curve because of the complication with disulfide bridges, aromatic groups, or prosthetic chromophores (13).

In the alpha-helix-forming trifluoroethanol, the $[m']$ for gliadin was $-7,500°$ at the 233 nm. minimum and 29,000° at the 198 nm. maximum. According to Yang (14) in his summation of main parameters of the ORD of polypeptides, $[m']$ is $-15,000°$ at 233 nm. and 75,000° at the 198 to 199 nm. peak for alpha-helix
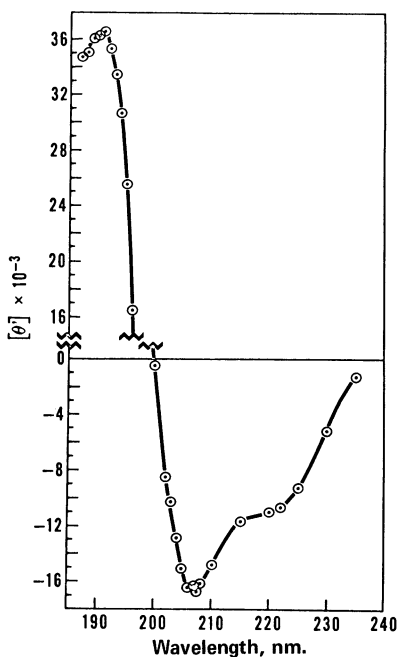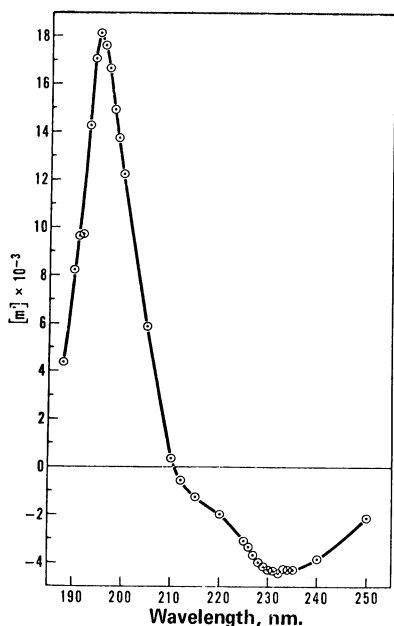
Fig. 1 (left). Far ultraviolet optical rotatory dispersion of wheat gliadin in 0.001N HCl at pH 3.1.

Fig. 2 (right). Circular dichroism of wheat gliadin in trifluoroethanol.

TABLE II. FAR ULTRAVIOLET PROPERTIES OF WHEAT GLUTENIN
AND GLIADIN

Protein	Solvent and (solution pH)	$[m']_{233}$	$[m']_{198}$	$[\theta']_{222}$	$[\theta']_{205-207}$	$[\theta']_{190}$
				Deg cm.^{2}/decimole		
Glutenin	0.001N HCl (3.1)	−4,200		−8,100±1,700	−13,700±2,000	
Gliadin	0.001N HCl (3.1)	4,300	+18,100[a]	−8,300± 700	−14,000±2,000	+11,900±2,000
	0.003N DCl (2.6)	−4,500		−10,200± 600	−18,400± 900	
	F$_3$CCH$_2$OH (4.0)	−7,500	+29,000	−10,600±1,800	−16,500± 800	+37,000±6,700

[a]Actual maximum at 195 nm.

conformation. Considering these data, the [m'] values for gliadin in trifluoroethanol reflect an increase in alpha-helicity when compared with those calculated from the 0.001N HCl solution. Further, alpha-helical content from ORD data was calculated from the values at the 233 nm. trough and the 198 nm. peak according to Yang (14) (Table III). These results are consistent with those computed from use of the modified Drude equation of Shechter and Blout (6,7).

The ORD results indicate that it is possible to change the conformations of glutenin and gliadin, largely by the proper choice of solvents. Interestingly, gliadin

TABLE III.   PERCENT ALPHA-HELIX OF GLUTEN PROTEINS

As Calculated from the Following Parameters[a]

Protein	Solvent	$[m']_{233}$	$[m']_{198}$	b_o	$[\theta]_{222}$	Average %
Glutenin	0.001N HCl pH = 3.1	17		15	16	16
Gliadin	0.001N HCl pH = 3.1	18	25	18	16	19
	0.003N DCl pH = 2.6	19		15	22	19
	F_3CCH_2OH pH = 4.0	42	42	32		39

[a]See text for details.

and glutenin can have a high proportion of alpha-helix even though they contain 17.6 and 13.4% proline residues, respectively, as calculated from amino acid data (15). The proline residue with its pyrrolidine ring does not fit into a right-handed alpha-helix. Proline residues are possibly not uniformly distributed in glutenin and gliadin molecules and, therefore, allow alpha-helix formation in those regions of the molecules that are free of proline. Such an uneven distribution of proline (0 to 27%) in fractions from an enzymatic hydrolysate of gliadin has been demonstrated by Finlayson (16).

The variability of b_o values of glutenin in 3M urea between pH 4 and 10 was previously noted by Wu and Cluskey (1). Since our previous work covered gluten protein behavior in 3M urea systems only, rotation measurements were made on glutenin in buffered solutions containing 8M urea and compared with unbuffered solutions containing 8M urea.

Taylor and Zobel[2] showed that $-b_o$ and $-a_o$ of glutenin in 8M unbuffered urea solutions varied with change in pH. From their data, it was apparent that glutenin in 8M urea at pH of 3.5 ± 1 was in a completely unordered form, whereas at higher pH values some alpha-helical structures remained. Although onset of denaturation of protein by urea may be time dependent (17), it was not observed for glutenin. The change in $-b_o$ (50 to 0) in going from slightly basic to acid conditions is accompanied by an increase in $-a_o$. In urea solutions, the transition in molecular structure which occurs in glutenin as a function of pH can be accounted for in terms of an alpha-helix-unordered structure transformation.

The 8M urea buffered solutions contained 0.11M KCl and 0.02M potassium phosphate. Measurements were made at both pH 7.8 and 5.4. At these pH values, b_o parameters were such that zero or near zero percentage alpha-helix was calculated. It should be noted that the a_o values were comparable to those from the unbuffered solutions.

The unfolding of glutenin by urea to the completely unordered conformation depends on the ability of urea to disrupt alpha-helices which, in turn, depends not only on urea concentration but also on pH. With a decrease in pH, 8M urea becomes a more effective agent for disrupting the alpha-helix. Since the isoionic point of glutenin is 7.1 as shown by Wu and Dimler (15), the glutenin molecule will

[2]N. W. Taylor and H. F. Zobel (unpublished results).

have little or no charge around pH 7 to 8 and will have a positive charge of around $40/10^5$ g. glutenin between pH 3 and 4. The positive charges on glutenin would tend to repel each other and thereby disrupt the alpha-helix. Although this charge density on glutenin does not appear very large, glutenin molecules have been shown to be sensitive to charge effect because of their polyelectrolyte nature [18].

Addition of 2-chloroethanol or urea to aqueous solutions of glutenin changes a_0 and b_0. These respective changes are compatible with either the formation or disruption of right-handed alpha-helices. The effects are reversible, and neither leads to the formation of irreversible aggregates.

The CD of gliadin and glutenin exhibited the characteristic maximum and minima of alpha-helical structure, i.e., minima at 222, 207, and maximum at 190 nm. Figure 2 shows the CD of wheat gliadin in trifluoroethanol, an alpha-helix-forming solvent. Below 235 nm., the spectrum is negative with a shoulder around 220 nm.; a negative minimum at 207 nm. then becomes positive toward a 191 nm. maximum. Crossover from negative to positive ellipticity occurs at 199 to 200 nm.

The mean residue ellipticity values corrected for refractive index, $[\theta']$, appear in Table II. These average ellipticity data were calculated from replicate CD determinations. The average deviation is given with each value. The $[\theta]$ values from CD of alpha-helical polypeptides are $-38,000°$ cm.^{2} per decimole at 222 nm., $-36,000°$ at 209 to 210 nm., and $+85,000°$ at 191 nm. [11]. These values are not corrected by refractive index whereas those in Table II have been. If no correction is applied, our values would be 5 to 8% higher.

The alpha-helical content can be estimated from $[\theta]_{222}$ by the expression of Hashizume et al. [19].

$$X^H = \frac{[\theta]_{222} - [\theta]_{222}^R}{-38,000°}$$

$[\theta]_{222}^R$ signifies the residue ellipticity of the protein in the disordered state. The average $[\theta]_{222}^R$ value for hemoglobin, lysozyme, and ribonuclease, $-2,900°$, was used in calculating the alpha-helical content. The alpha-helix content for gliadin in trifluoroethanol was not determined because the model for calculation was developed with an aqueous medium. The $[\theta]_{222}$ results obtained from gliadin and glutenin in 0.001N HCl, pH 3.1, are similar to those reported for alpha-gliadin by Kasarda et al. [12]. They reported an average $[\theta]_{222}$ of $-9,680°$. The $[\theta]_{222}$ for gliadin is $-9,000°$ and that for glutenin $-9,100°$ with average deviations being $\pm800°$ and $\pm1,900°$. Our results support the conclusion of Kasarda et al. [12] that the many components of a gliadin mixture have conformations quite similar to those of their alpha-gliadin components.

The amount of alpha-helix in gliadin and glutenin is compared in Table III. Most of the data result from ORD spectra and some from CD. The calculation of alpha-helix using $[m']_{233}$ and $[m']_{198}$ is from the method of Yang [14]. Values of alpha-helix from the Moffitt-Yang equation using b_0 also appear in the table. For the dilute acid solutions, $b_0 = -630$ for 100% alpha-helix, $b_0 = 0$ for 0% alpha-helix; for the trifluoroethanol solution, $b_0 = -816$ for 100% alpha-helix [8].

TABLE IV.   AMIDE I FREQUENCIES OF WHEAT GLIADIN
AND OTHER PROTEINS

| Protein | Solvent | Amide I Max. Frequency $(cm.^{-1})$ | |
		Exp. Values	Lit. Values[a]
Myoglobin (α-helix)	F_3CCH_2OH	1,652	1,650
α-Casein (unordered)	$(CF_3)_2CO \cdot 1\frac{1}{2} H_2O$	1,660	1,658
Gliadin	$(CF_3)_2CO \cdot 1\frac{1}{2} H_2O$	1,665, 1,663	
Gliadin	F_3CCH_2OH	1,658	
Gliadin	0.003N DCl in D_2O	1,646, 1,644	

[a]Timasheff et al. (20).

The calculation of alpha-helicity from CD by the method of Hashizume et al. (19) has been discussed earlier.

The possibility of conformations other than alpha-helix and unordered types in gliadin has been investigated. A comparison was made of our CD data with those from a number of well-known proteins possessing other order conformations (20). This appraisal and a calculation of model synthetic mixtures of alpha-helix, unordered, and other ordered conformations, i.e., poly-L-proline I and II, showed that neither poly-L-proline I nor II types is likely to be present within the accuracy of the measurement. The possible presence of beta-structure is discussed below.

IR absorption spectra in the Amide I band region were obtained on wheat gliadin in DCl in D_2O and fluorinated solvents. Two proteins of known conformations were also examined and served as models for comparative band assignments (Table IV). They were: myoglobin representing the alpha-helical structure and alpha-casein representing the unordered structure. These reference proteins were selected rather than model synthetic polypeptides. Susi et al. (21) have shown that synthetic polypeptides in D_2O solution are not a reliable guide for the corresponding maximum IR absorptions of globular proteins. The synthetic polypeptides give different frequencies for the same conformation as a result of the nature of the side chains present. The literature and experimental values of the model compounds' maximum frequency also appear in Table IV. The maximum frequency values of myoglobin and alpha-casein which we obtained agree well with the solid film values for alpha-helix and unordered conformations as given by Timasheff et al. (20).

Since water has a strong absorption band at 1,650 cm.$^{-1}$, the frequency of the Amide I band, measurements were made in DCl in D_2O and organic solvents. In 0.003N DCl, the maximum for gliadin at 1,644 to 1,646 cm.$^{-1}$ is closer to that of the unordered structure at 1,643 cm.$^{-1}$ than that of alpha-helix at 1,650 cm.$^{-1}$, as reported by Timasheff et al. (20). This observation suggests the presence of some alpha-helix, together with a large amount of unordered structure. In this solvent no evidence of significant change in frequency maximum occurred during 4 days. Essentially, unordered structure was in evidence when gliadin in hexafluoroacetone

sesquihydrate was compared with alpha-casein. Apparently the fluorinated solvent has shifted the maximum frequency of gliadin several wave numbers above 1,660. Further, the maximum frequency of 1,658 cm.$^{-1}$ for gliadin in trifluoroethanol is between that of the alpha-helical model, myoglobin, at 1,652 cm.$^{-1}$ and that of unordered structure at 1,660 to 1,665 cm.$^{-1}$ and suggests the presence of alpha-helical and unordered structure in gliadin. Since no peak or shoulder for beta-structure in the Amide I region (1,632 cm.$^{-1}$) was visible, the amount of beta-structure was not large, but the possibility of a small amount of beta-structure is not eliminated.

IR data add further support to the interpretation that gliadin consists chiefly of alpha-helical and unordered structures.

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