Sorghum Prolamins: Their Optical Rotatory Dispersion, Circular Dichroism, and Infrared Spectra

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The molecular conformation of sorghum prolamin was explored because of its high levels of nonpolar amino acids, its strong tendency to gel, and its poor solubility. Conformations of prolamin from four hybrids of grain sorghum were studied by optical rotatory dispersion (ORD), circular dichroism (CD), and infrared spectra in several solvents. Infrared absorption spectrum of prolamin in 60% *tert*-butyl alcohol (*tert*-BuOH) and D_2O showed the presence of α-helix and unordered structure, as well as the absence of β-structure. ORD data of the prolamins in 60% *tert*-BuOH give α-helix content of 40–47% independent of hybrids and of color of the prolamin solution. The α-helix content of the prolamins in 60% *tert*-BuOH + 1.5 *M* guanidine hydrochloride (G–HCl) is lowered somewhat to 34–40%, but is greatly reduced in 6 *M* G–HCl. The CD and far ultraviolet ORD curves of decolorized prolamin give α-helix content in agreement with that from ORD data. The high level of α-helix for sorghum prolamin supports the concept that hydrogen bonds between backbone polypeptide chains are protected from aqueous or other polar residues by a nonpolar environment resulting from the side chains.

Although grain sorghum is an important crop in the United States as well as in the world, little is known about its proteins. Amino acid composition of grain sorghum proteins has been reported by Virupaksha and Sastry (1968) and by Jones and Beckwith (1970). Electrophoretic patterns of grain sorghum proteins were studied also by Sastry and Virupaksha (1967, 1969) and by Jones and Beckwith (1970). The prolamin accounts for about 40% of total protein from sorghum and has high levels of nonpolar amino acids and is very water-insoluble. The tendency of sorghum prolamin solution to gel is of special interest both from theoretical and practical points of view. The very poor solubility of the sorghum prolamin severely limits the solvents to 60% *tert*-butyl alcohol (*tert*-BuOH), guanidine hydrochloride (G–HCl), or a combination of the two. This tendency of the prolamin to gel suggests that a unique conformation may be present in the prolamin. This paper reports the effect of pigment, solvent, and gelling on the conformation of prolamins in four grain sorghum hybrids by optical rotatory dispersion (ORD), circular dichroism (CD), and infrared measurements.

MATERIALS AND METHODS

Isolation of Protein. The four grain sorghum hybrids were OK612, RS626, TE77, and Funk G-766. OK612 is a heteroyellow endosperm hybrid, RS626 and TE77 are typical hybrids with considerable color, and Funk G-766 has a white endosperm. The sorghum prolamins were extracted from defatted sorghum flour by 60% *tert*-BuOH after salt and water-soluble proteins were first removed by the method of Jones and Beckwith (1970). Some colored prolamin solutions were decolorized by activated carbon before use.

ORD. In general, optical rotation measurements were made in a Cary 60 recording spectropolarimeter (Cary Instruments) at 26° C from 600 to around 340 mμ. For some proteins, measurements were also made from around 360 to 250 mμ. The concentration of prolamins varied between 0.2 and 1.9% and the path lengths of cells used were such that the maximum absorbance of the solution in the wavelength range measured was less than 2. In the far ultraviolet–ORD measurement the wavelength ranged from 250 to 195 mμ in a 0.1-cm cell. A constant band pass of 1.5 mμ was used throughout. The instrument was calibrated as described previously (Wu and Cluskey, 1965). The solvent blank was subtracted from the observed rotation of the protein solution. The average of two or more runs was used.

Both visible and near ultraviolet–ORD data were plotted by two methods. The first was according to Moffitt (1956)

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

where M is the average residue weight, which is 114 for sorghum prolamin calculated from the amino acid composition, and n is refractive index of the solvent. The wavelength of the n measurement is 589 mμ, and the dispersion of n is not considered. The value of λ_0 was taken to be 212 mμ, which was found best for poly-γ-benzyl-L-glutamate in a variety of solvents (Moffitt and Yang, 1956). The parameter b_0 was calculated from $\frac{M}{100}\left(\frac{3}{n^2+2}\right)\Big/\lambda_0^4$ times the slope of the straight line of $[\alpha](\lambda^2-\lambda_0^2)$ *vs.* $1/(\lambda^2-\lambda_0^2)$. Data were also plotted according to a modified one-term Drude equation (Yang and Doty, 1957).

$$\lambda^2[\alpha] = \lambda_c^2[\alpha] + k$$

The parameter λ_c was obtained from the slope of a plot of $\lambda^2[\alpha]$ *vs.* $[\alpha]$.

CD. The CD measurements were carried out in a Cary model 6001 CD accessory for the Cary 60 instrument in a 0.1-cm cell from 250 to 195 mμ at 26° C. The solvent blank was subtracted from the observed ellipticity of the protein solution. The average of two or more runs was used.

Infrared Spectrum. Infrared absorption spectra were measured with a Perkin-Elmer 621 grating infrared spectrophotometer. Calcium fluoride cells of 0.1-mm path length were used with decolorized OK612 sorghum prolamin in 60% *tert*-BuOH in D_2O. The infrared measurements were made against a solvent blank on the absorbance scale with an expanded wavelength setting between 1700 and 1450 cm^{-1}. The wavelength scale of the spectrophotometer was calibrated by water vapor. Concentration of prolamin was around

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Table I. $E^{1\%}_{1cm}$ and Characteristics of Ultraviolet Spectra of Sorghum Prolamins

Hybrid	Solvent and color	277 mμ Max	320 mμ Max	320 mμ Not max	350 mμ
RS626	60% *tert*-BuOH, pink	12.0	5.62		3.10
	60% *tert*-BuOH + 1.5 *M* G-HCl, pink	12.2	5.79		3.20
	15% *tert*-BuOH + 5.8 *M* G-HCl, pink	11.9	5.08[a]		2.68
	60% *tert*-BuOH, carbon decolorized	7.81		0.59	0.40
	60% *tert*-BuOH + 1.5 *M* G-HCl, carbon decolorized	7.96		0.56	0.35
OK612	60% *tert*-BuOH, slightly yellow	11.0	4.65		2.44
	60% *tert*-BuOH, carbon decolorized	7.99		0.77	0.48
	60% *tert*-BuOH + 1.5 *M* G-HCl, carbon decolorized	8.08		0.84	0.48
Funk G-766	60% *tert*-BuOH, slightly yellow	11.3	4.06		2.05
	60% *tert*-BuOH + 1.5 *M* G-HCl, slightly yellow	11.1	3.98		2.00
TE77	60% *tert*-BuOH, pink	10.8	3.90		2.25
	60% *tert*-BuOH + 1.5 *M* G-HCl, pink	10.8	3.94		2.19

[a] A shoulder instead of a maximum was observed.

Table II. Visible and Near Ultraviolet Optical Rotatory Properties of Sorghum Prolamins at 26° C

Hybrid	Solvent and color	$[\alpha]_{589}$ (deg)	λ_c (mμ)	b_0 (deg)	Percentage α-helix[a]
OK612	60% *tert*-BuOH, slightly yellow	−35	282	−304	44
	60% *tert*-BuOH, decolorized, pH 5.99	−40	268	−289	41
Funk G-766	60% *tert*-BuOH, slightly yellow, pH 6.49	−35	281	−296	42
TE77	60% *tert*-BuOH, pink, pH 6.61	−32	256	−326	47
RS626	60% *tert*-BuOH, pink, pH 6.66	−37	271	−278	40
	60% *tert*-BuOH, decolorized	−29	285	−297	43
OK612	60% *tert*-BuOH + 1.5 *M* G-HCl,[b] decolorized, pH 4.59	−49	266	−243	36
Funk G-766	60% *tert*-BuOH + 1.5 *M* G-HCl, slightly yellow, pH 4.55	−49	256	−234	34
TE77	60% *tert*-BuOH + 1.5 *M* G-HCl, pink, pH 4.6	−46	260	−236	35
RS626	60% *tert*-BuOH + 1.5 *M* G-HCl, pink, pH 5.40	−46	270	−274	40
	60% *tert*-BuOH + 1.5 *M* G-HCl, decolorized, pH 4.66	−49	266	−241	36
RS626	44% *tert*-BuOH + 5.5 *M* G-HCl, pink	−97	222	−60	9
	36% *tert*-BuOH + 5.7 *M* G-HCl, decolorized	−92	229	−90	14
Funk G-766	6 *M* G-HCl, slightly yellow	−142	219	−35	5

[a] The percentage α-helix is $-b_0/6.98$ for 60% *tert*-butyl alcohol (*tert*-BuOH), $-b_0/6.8$ for 60% *tert*-BuOH + 1.5 *M* G-HCl, $-b_0/6.38$ for 44% *tert*-BuOH + 5.5 *M* G-HCl and for 36% *tert*-BuOH + 5.7 *M* G-HCl, and $-b_0/6.53$ for 6 *M* G-HCl. For details see text. [b] G-HCl = guanidine hydrochloride.

1.5%. The solvent *vs.* solvent blank was subtracted from the observed absorbance of the protein solution. The average of two or more runs was used.

Ultraviolet Spectrum. Ultraviolet spectra were measured in a Cary 14 recording spectrophotometer against solvent. The solvent *vs.* solvent blank was subtracted from the observed absorbance of the protein solution. The average of two or more runs was used.

Refractive Index. The indices of refraction, n, of the various solvents were determined with a Bausch & Lomb refractometer thermostated at 25° C.

Concentration determination of prolamin was made from

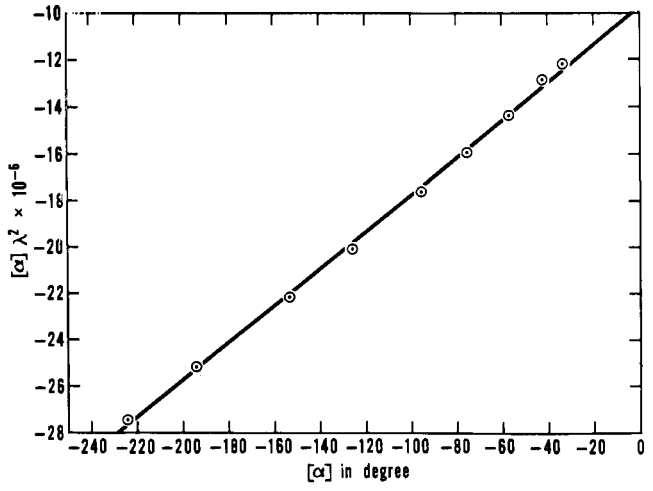

Figure 1. One-term Drude plot of OK612 sorghum prolamin in 60% *tert*-butyl alcohol (*tert*-BuOH). Protein concentration, 1.09%; 1-cm cell, 600–350 mμ

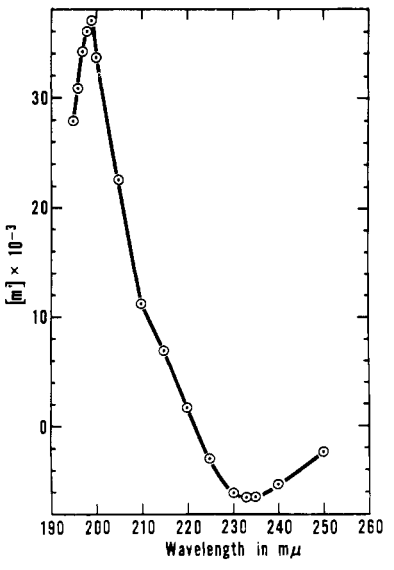

Figure 3. Far ultraviolet–optical rotatory dispersion of decolorized OK612 sorghum prolamin in 60% *tert*-BuOH. Protein concentration, 0.0189%; 0.1-cm cell, pH 5.45

micro-Kjeldahl nitrogen analysis for *tert*-BuOH solution. Ultraviolet absorption spectrum of each prolamin was standardized against the micro-Kjeldahl nitrogen analysis (weight of prolamin is equal to $N \times 6.25$). The ultraviolet absorption spectrum of each prolamin was not affected by the presence of G–HCl in *tert*-BuOH and the same ultraviolet conversion factor applied. Therefore, ultraviolet absorption was selected to determine the prolamin concentration in solution containing G–HCl because micro-Kjeldahl analysis cannot be made in the presence of G–HCl.

The pH values were measured with a Radiometer pH meter 4 or a Corning Model 12 pH meter. Commercially available chemicals of the highest purity were used in general without further purification.

RESULTS

The $E^{1\%}_{1cm}$ and characteristics of ultraviolet spectra of sorghum prolamins are shown in Table I. The ultraviolet spectra of sorghum prolamins depend somewhat on the particular hybrid and considerably on color. The $E^{1\%}_{1cm}$ value at the 277 mμ absorption maximum is the same for any particular prolamin in 60% *tert*-BuOH, 60% *tert*-BuOH plus 1.5 *M* G–HCl, or other *tert*-BuOH and G–HCl combination. The colored solutions have appreciable $E^{1\%}_{1cm}$ value at 350 mμ and have an additional flat absorption maximum at 320–324 mμ. When the prolamin solutions were decolorized by passing the solutions through activated carbon, the flat absorption maximum at 320–324 mμ disappeared, the $E^{1\%}_{1cm}$ values decreased drastically at 350 mμ, and the respective $E^{1\%}_{1cm}$ values of RS626 and OK612 at 277 mμ decreased to about two-thirds of those of the colored solutions.

The visible and near ultraviolet–ORD data on sorghum prolamins in 60% *tert*-BuOH, 60% *tert*-BuOH plus 1.5 *M* G–HCl, 6 *M* G–HCl, and other *tert*-BuOH and G–HCl combinations are summarized in Table II. A typical one-term Drude plot of sorghum prolamin is shown in Figure 1; a typical Moffitt plot is shown in Figure 2.

A far ultraviolet–ORD curve of decolorized OK612 sorghum prolamin in 60% *tert*-BuOH is plotted in Figure 3. This curve shows a minimum at 233 mμ and a maximum at 199 mμ.

The CD spectrum of the decolorized OK612 sorghum prolamin in 60% *tert*-BuOH appears as Figure 4. The curve

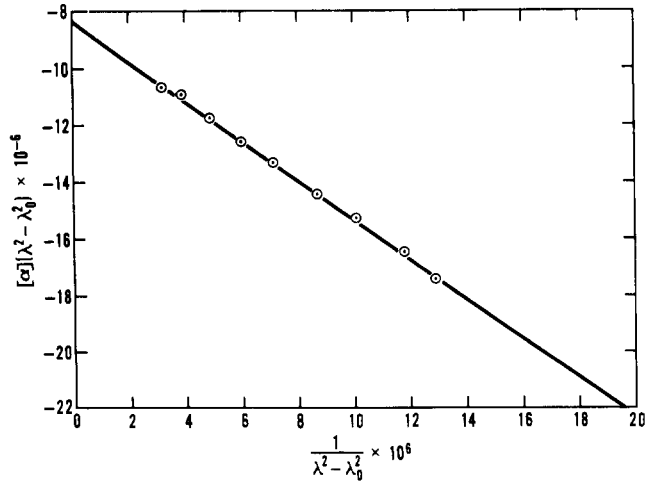

Figure 2. Moffitt plot for OK612 sorghum prolamin in 60% *tert*-BuOH. Protein concentration, 1.09%; 1-cm cell, 600–350 mμ

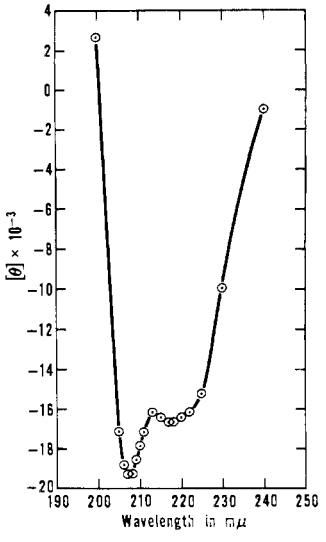

Figure 4. Circular dichroism spectrum of decolorized OK612 sorghum prolamin in 60% *tert*-BuOH. Protein concentration, 0.0189%; 0.1-cm cell, pH 5.45

shows two minima at 217–218 mμ and at 207–208 mμ, and it becomes positive below 201 mμ. The mean residue ellipticity value, [θ], is 36,600 at 195 mμ and $[\theta]_{195}$ is not included in Figure 4 in order to give an enlarged figure between 200 and 240 mμ. The lowest wavelength attainable with this solvent in a 0.1-cm cell is 193 mμ because excessive absorption of the solvent below 193 mμ makes measurement impossible.

The infrared spectrum of decolorized OK612 sorghum prolamin in 60% *tert*-BuOH in D_2O had an absorption maximum between 1645 and 1651 cm^{-1}. There was no other peak or shoulder between 1700 and 1600 cm^{-1}.

DISCUSSION

The infrared spectrum of decolorized OK612 sorghum prolamin can be compared with the infrared spectra of proteins of known conformations in D_2O. Timasheff *et al.* (1967) reported that the maximum absorption frequency for unordered structure is 1643 cm^{-1}, for α-helix is 1650 cm^{-1}, and for β-structure is 1632 cm^{-1}. The observed maximum absorption frequency of 1645–1651 cm^{-1} and the absence of a peak or a shoulder at or near 1632 cm^{-1} for sorghum prolamin indicate that the protein is a mixture of α-helix and unordered structure and that there is no evidence of the presence of any β-structure.

The far ultraviolet-ORD of sorghum prolamin in 60% *tert*-BuOH revealed the typical α-helix minimum at 233 mμ and the typical α-helix minimum at 199 mμ. The magnitude of the maximum at 198 mμ and the minimum at 233 mμ can serve to estimate the amount of α-helix. The fraction of α-helix can be calculated by $-([m']_{233} + 2000)/13{,}000$ and by $([m']_{198} + 5000)/80{,}000$ (Yang, 1967). The calculated values of α-helix are 51% from $[m']_{198}$ and 34% from $[m']_{233}$. Since the 198-mμ peak and the 233-mμ trough are influenced by aggregation (Yang, 1967) and since the prolamin in 60% *tert*-BuOH will gel in a few hours (suggesting aggregation of protein), the calculated α-helix values from $[m']_{198}$ and $[m']_{233}$ are probably less accurate than those from the Moffitt parameter b_0.

The CD of α-helix polypeptide has minima at 207 and 221–222 mμ. The CD spectrum of sorghum prolamin in Figure 4 shows minima at 207–208 and 217–218 mμ. The ellipticity value at 221 mμ is quite close to that at 218 mμ, and the difference may reflect experimental uncertainty. The spectrum in Figure 4 may therefore be considered as that of α-helix (the maximum at 190–191 mμ for α-helix cannot be observed because the solvent absorbs excessively at 193 mμ). The α-helical content can be estimated by $([\theta]_{222} + 2900)/-38{,}000$ (Hashizume *et al.*, 1967). An α-helical content of 35% is obtained for the prolamin.

Since the infrared spectrum of sorghum prolamin does not show any evidence of β-structure, the ORD and CD spectra are typical of α-helix; furthermore, since the CD spectra of poly-L-proline I and II (Timasheff *et al.*, 1967) are different from the observed CD spectrum, the sorghum prolamin is likely a mixture of α-helix and unordered structure. The visible and near ultraviolet-ORD data were therefore analyzed by the Moffitt equation to obtain α-helix contents without any complication from other known structures. Cassim and Taylor (1965) reported that the refractive index of solvent influences the value of b_0 for 100% α-helix and that $-b_0 = 1701 - 730.3\,n$ for 100% α-helix, where n is the refractive index. The values of b_0 for 100% α-helix from this equation are −698 for 60% *tert*-BuOH, −680 for 60% *tert*-BuOH plus 1.5 *M* G-HCl, −638 for 44% *tert*-BuOH plus 5.5 *M* G-HCl and for 36% *tert*-BuOH plus 5.7 *M* G-HCl, and −653 for 6 *M* G-HCl. These values of b_0 for 100% α-helix are the basis from which the percentage α-helix in Table II was calculated. If the b_0 value for aqueous solution of −630 is chosen for 100% α-helix, the percentage α-helix will be somewhat higher. Regardless of the b_0 values for 100% α-helix, the general conclusion will be the same.

Table III. The α-Helix Content of Decolorized OK612 Sorghum Prolamin in 60% *tert*-BuOH by Different Methods

Method and wavelength range	Percentage α-helix[a]
Moffitt b_0, 600–305 mμ	41
Moffitt b_0, 340–250 mμ	35
$[m']_{233}$	34
$[m']_{198}$	51
$[\theta]_{222}$	35
Average	39

[a] Percentage α-helix is $-b_0/6.98$, $-([m']_{233} + 2000)/13{,}000$, $([m']_{198} + 5000)/80{,}000$, and $-([\theta]_{222} + 2900)/38{,}000$. For details see text.

Table II shows that the sorghum prolamins in 60% *t*-BuOH have a considerable amount of α-helix from 40 to 47%. There is no significant difference between hybrids and between colored and decolorized samples as far as percentage α-helix is concerned. The rather high λ_c values also are consistent with the percentage α-helix in 60% *t*-BuOH. The percentage α-helix decreased somewhat to 34–40% for sorghum prolamins in 60% *t*-BuOH plus 1.5 *M* G-HCl, and the $-[\alpha]_{589}$ values increased from 29 to 40° to 46 to 49°. The generally lower λ_c values in 60% *t*-BuOH plus 1.5 *M* G-HCl also reflect the change in $[\alpha]_{589}$ and percentage α-helix. Again, no significant difference in optical rotatory properties is observed between hybrids and between colored and decolorized samples. When G-HCl concentration increased from 1.5 *M* to 5.5–6 *M*, the percentage α-helix and λ_c values are greatly reduced, and the $-[\alpha]_{589}$ values increased significantly.

Since sorghum prolamins will gel in 60% *tert*-BuOH, a study of ORD *vs.* time was made for TE77 prolamin. Four successive runs were made on the freshly extracted prolamin solution in 60% *tert*-BuOH for 3 hr, after which time a gel formed. No difference was observed in the ORD results between the four runs. Therefore, the gelling process does not influence α-helix content or conformation of the prolamin.

The high α-helix content of sorghum prolamin in 60% *tert*-BuOH raised the possibility that *tert*-BuOH might be a helix-forming solvent. Ponca wheat gliadin in 60% *tert*-BuOH was used in ORD and the same Moffitt method gave an α-helix content of 25%. The α-helix content of the same Ponca gliadin in 0.002 *N* HCl was 24% (Wu and Cluskey, 1965). Therefore *tert*-BuOH is probably not a helix-forming solvent in general, and the high α-helix contents are likely inherent to the sorghum prolamins themselves.

The α-helix content of decolorized OK612 sorghum prolamin in 60% *tert*-BuOH by different methods is listed in Table III. The average value of 39% is within experimental error of the 41% value shown in Table II.

The sorghum prolamin has a high level of nonpolar amino acid (the sum of proline, glycine, alanine, valine, isoleucine, leucine, and phenylalanine is 59%), a high level of glutamine (30% of glutamic acid largely in the form of glutamine), and a low level of polar amino acids (Jones and Beckwith, 1970). The abundant glutamine residues provide good opportunity for hydrogen bonding. It is somewhat surprising to find that sorghum prolamin can have 40–47% α-helix because the prolamin has 10% proline and proline residue does not fit into α-helix. The high levels of both α-helix and proline

would indicate that either the α-helix segments are relatively short or the proline residues are not uniformly distributed along the prolamin molecule. This would allow a comparatively long segment of α-helix to form in the regions relatively free of proline. The solubility characteristics of the sorghum prolamin indicate that the nonpolar residues may be oriented outward unlike those in water-soluble proteins.

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