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Physical Chemical Characterization of Grain Sorghum Prolamine Fractions and Components

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The prolamines of grain sorghum have the unique property of forming gels at low protein concentrations in a variety of solvents. Differences in the solubility properties and amino acid composition of prolamine fractions are described, and evidence is presented to show that protein from sorghum prolamines undergoes noncovalent interaction even in such solvents as 6 *M* guanidine hydrochloride.

The proximate composition and proteins of three American grain sorghum hybrids were previously described (Jones and Beckwith, 1970). We noted that the prolamine class of protein from these hybrids readily formed gels in alcohol–water systems, as well as in such solvents as dimethyl sulfoxide and 8 *M* urea solutions. In the alcohol–water system the addition of 1.5 *M* guanidine hydrochloride (GHCl) prevented gel formation, whereas the addition of sulfhydryl blocking agents or disulfide bond-breaking compounds did not influence the gelling phenomena.

As a continuing study of the properties of these proteins, we wish to report observed differences in solubility properties and amino acid composition between two fractions from the prolamines of the three hybrids used earlier. In addition, the sedimentation equilibrium behavior of these isolates was examined as a function of protein concentration and pH in solutions of 6 *M* GHCl which is considered to be strong noncovalent bond-disrupting agent.

MATERIALS AND METHODS

For quantitative measurements, the GHCl used was an ultra-pure grade obtained from Mann Research Laboratories. All other standard chemicals used in preparing solutions or solvents were reagent grade.

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PROTEIN ISOLATION

The method used to extract the prolamines from grain sorghum flour has been presented earlier (Jones and Beckwith, 1970). The prolamine fraction is dispersed in 95% ethanol and then cooled to 9–10°C. After centrifugation at this temperature, the solution is treated with decolorizing carbon to remove red pigments. Evaporation of the alcohol yields a white protein preparation used as one fraction in these studies.

The fastest migrating gel electrophoretic component of the prolamines (Jones and Beckwith, 1970) was isolated from a 0.5% w/v solution of decolorized prolamine in 60% v/v *tert*-butanol–water containing 1.5 *M* GHCl to prevent gelling. Water (1.8 vol) is slowly added to the solution at room temperature. After centrifuging at about 10,000 $\times$ *g*, the centrifugate is dialyzed against water and then freeze-dried. The crude product is taken up in 6 *M* GHCl (8–10% w/w total protein concentration) and passed over a 96 $\times$ 5 cm Sephadex G-150 column at 25°C with a flow rate of 10 ml per hour using 6 *M* GHCl as eluent. The first 1020 ml of effluent are discarded and the next 200 ml are collected, exhaustively dialyzed against water, and then freeze-dried.

Amino Acid Analysis and Electrophoresis. The methodology was described in our earlier report (Jones and Beckwith, 1970).

Ultracentrifugation. A Spinco Model E ultracentrifuge equipped with RTIC control, photoelectric scanner attachment, multiplexing accessory, and spherical mirror optical system was used to examine sedimentation equilibrium be-

havior of the prolamine fractions at 25°C. All scanner absorbance profiles were made at 278 nm. A six-place aluminum rotor was used to carry out multiple analyses during single sedimentation experiments. An automated data gathering system and an IBM 1130 computing system, to be described elsewhere (Beckwith *et al.*, 1971), were used to collect and analyze the data in scanner profiles at sedimentation equilibrium.

For sedimentation analyses, protein stock solutions were prepared in neutral 6 *M* GHCl, 6 *M* GHCl containing 0.1 *M* citric acid, or 6 *M* GHCl containing 0.001 *N* HCl. All stock solutions were equilibrated by dialysis at 4°C against solvent prior to making dilutions with equilibrated solvents.

An apparent molecular weight at any radial position within the solution column at equilibrium is calculated using the accepted definition.

$$\bar{M}_{\mathrm{w\,app}} = \frac{2RT}{(1 - \bar{v}\rho)_{\mathrm{w}}^{2}} \frac{d\ln c}{d(r^{2})} \quad (1)$$

Terms on the right in eq 1 have the standard meanings. Values for $\bar{v}$ were calculated from amino acid data (McMeekin *et al.*, 1949) while the value of ρ for all 6 *M* GHCl solutions was found to be very close to 1.14 g/cm³ (Reisler and Eisenberg, 1969).

The weight average molecular weight for the nonredistributed protein solute was calculated in the computer program (Beckwith *et al.*, 1971) by the relationship

$$\bar{M}_{\mathrm{w}} = \int_{r_{\mathrm{m}}}^{r_{\mathrm{b}}} \bar{M}_{\mathrm{w\,app}}(r)C(r)r\,dr \Big/ \int_{r_{\mathrm{m}}}^{r_{\mathrm{b}}} C(r)r\,dr \simeq \sum_{i=1}^{n} \bar{M}_{\mathrm{w\,app}}(r_i)\,C(r_i)r_i \Big/ \sum_{i=1}^{n} C(r_i)r_i \quad (2)$$

which does not require prior information about the total concentration at zero time. The index n in eq 2 has a value of about 125–150 between the radial position of the meniscus (r_{m}) and the bottom of the sector cell (r_{b}). The quantity $C(r_i)$ is the total concentration of macromolecular solute or any quantity directly proportional to the total concentration at some radial position r_i within the liquid column.

RESULTS AND DISCUSSION

Since the properties to be discussed in this report showed no apparent dependence upon the particular grain sorghum hybrid used, the major portion of the results presented was obtained using a single hybrid (TE-77).

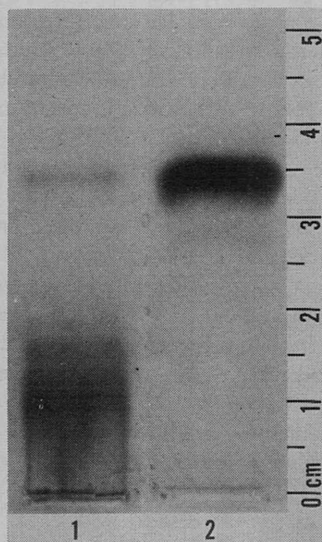

Figure 1. Electrophoretic patterns of protein from TE-77 prolamines. 1. Protein soluble in 95% ethanol at 9°C. 2. Fastest migrating component of proteins from prolamine fractions

Table I. Amino Acid Contents of TE-77 Prolamine and Prolamine Isolates

	g/16 g Nitrogen		
Amino acid	Whole prolamine	95% ethanol fraction	Fast component
Lysine	0.1	0.0	0.0
Histidine	0.9	0.9	0.0
Arginine	1.0	1.2	4.3
Aspartic	6.5	5.9	2.9
Threonine	2.6	2.3	3.8
Serine	4.1	4.0	3.3
Glutamic	30.0	24.8	24.9
Proline	10.0	5.8	8.1
Glycine	1.1	1.1	3.4
Alanine	12.4	10.6	9.9
Half-cystine	0.4	0.0	6.3
Valine	5.0	6.9	4.7
Methionine	1.0	0.6	10.0
Isoleucine	4.8	3.8	1.4
Leucine	19.2	16.2	11.5
Tyrosine	5.5	4.5	7.8
Phenylalanine	6.4	5.2	1.7
Ammonia	3.5	3.8	2.8

For the three hybrids studied, about 55% of the prolamine nitrogen remains soluble in 95% ethanol at 9°C. While decolorizing with carbon removes about 7% of the prolamine nitrogen, we have no evidence that this loss involves the selective removal of a particular prolamine component (Jones and Beckwith, 1970). The 95% ethanol-soluble proteins do not form gels in this solvent, but will do so, however, in those solvents mentioned earlier.

The single component isolate used in these studies constitutes about 0.4% of the total weight of the prolamine fraction. At comparable concentrations, solutions of the single component do not gel as do solutions of the original mixture.

Typical gel electrophoretic patterns for the 95% ethanol-soluble proteins and single component isolates are shown in Figure 1. The ethanol-soluble mixture contains somewhat less material remaining at the origin than does the whole prolamine fraction (not shown); but otherwise the gel pattern for this preparation is indistinguishable from that for the whole prolamine. The pattern for the single component appears on the right in Figure 1. This component is also present in the ethanol-soluble mixture. What is probably lost in the reproduction of this pattern is the appearance of three much fainter bands behind the intense 3.5 cm band. The stronger of the three bands is at 2.8 cm and the other much fainter bands are at 2.0 and 1.4 cm, approximately. Evidence to be presented indicates that these bands possibly arise from association products of a monomer unit.

The amino acid compositions for the whole prolamine, the ethanol-soluble fraction, and the single component from the TE-77 hybrid appear in Table I. Ethanol fractionation does not grossly alter the amino acid composition of the preparation with the exception of proline content. The single component has several significant differences in amino acid content in that it contains no detectable lysine or histidine, but relatively large amounts of arginine. On a comparative basis this single component contains a disproportionately large amount of the sulfur-containing amino acids. The high methionine content is unique for proteins from cereal grains; however, polypeptides from crambe do have unusually high cysteine values (Van Etten *et al.*, 1965).

The amount of free ammonia obtained during the amino acid analysis is shown in Table I to indicate that the aspartic and glutamic acid residues probably exist chiefly in the amide form in all three preparations. Like other cereal prolamines, these preparations are also characterized by their large amounts of residues possessing aliphatic side chains.

To minimize pressure effects and to eliminate the possibility of solvent redistribution during sedimentation analyses, 6 M GHCl was the best solvent in which to compare the sedimentation equilibrium behavior of the single component and the 95% ethanol-soluble protein. From the results shown in Figure 1 it is quite likely that the ethanol solubles would constitute a paucidispersed system in the ultracentrifuge. Therefore, the main purpose for examining such a possible mixture in the ultracentrifuge was to note changes in the weight average molecular weight for the nonredistributed solute at different pH values and initial protein concentration. It was assumed that a sedimentation analysis of the 3.5 cm band in Figure 1, under similar experimental conditions as employed with the mixture, would indicate a minimum molecular weight value for the proteins of sorghum prolamines.

Table II presents the weight average molecular weight values obtained in neutral GHCl for the 95% ethanol-soluble mixture as a function of initial protein concentrations. For the most concentrated solution shown in Table II, the apparent weight average molecular weight ranged from 36,400 at the meniscus to 165,000 at the bottom of the cell, and for the most dilute solution these values were 22,300 and 140,000, respectively. It has been estimated that the precision of a single weight average molecular weight value is ±2.5%. The molecular weight values given in Table II show a definite decrease with decreasing initial protein concentration. As a first approximation, a linear extrapolation of M_w^{-1} values obtained from Table II to infinite dilution would indicate a minimum weight average molecular weight value of about 36,000 ± 4500.

When the 6 M GHCl solvent was made acidic with either 0.1 M citric acid or 0.001 N HCl, $\overline{M}_w$ values showed no change with changes in initial protein concentrations. For six protein solutions in acidic 6 M GHCl ranging in protein concentrations from 0.487 to 0.152 mg/ml, the $\overline{M}_w$ value was 85,200 ± 5200. The average $\overline{M}_{w\,app}$ values ranged from 42,300 at the meniscus to 380,000 at the bottom of the cell. Clearly these results, when compared to those obtained in neutral solution, indicate that sorghum prolamine proteins can likely undergo strong noncovalent interactions to form association product, as well as being a paucidispersed system. In neutral solutions the presence of a few ionized carboxyl groups acting as solvent interaction sites might be sufficient to induce dissociation of larger units as the protein concentration decreases.

For the single component (3.5 cm band in Figure 1), the equilibrium molecular weight showed no dependence upon pH at 25°C in 6 M GHCl solutions. Molecular weight values for this component at four different initial protein concentrations are shown in Table III. For the two most dilute solutions in Table III, the apparent molecular weight was constant across the liquid columns, indicating that in these solutions the protein was sedimenting as an ideal solute. For the next most concentrated solution, the $\overline{M}_w$ values in Table III described the apparent molecular weight over at least 90% of the liquid column. The analysis, however, did indicate that some higher molecular weight material was present very near to the bottom of the sector cell. The computer analysis for the most concentrated solution shown in Table III clearly indicates that the molecular weight changes with position in the liquid column. For this solution, $\overline{M}_{w\,app}$ values ranged from 20,000 at the meniscus to 43,600 at the bottom of the cell. This range of molecular weights, in addition to the appearance of minor bands in the gel electrophoretic pattern (Figure 1), could be interpreted as evidence supporting at least a monomer–dimer association–dissociation reaction at sufficiently high protein concentrations. On the basis of the amino acid composition given in Table I for the single component, a single chain peptide containing two isoleucine residues would have a molecular weight of 17,100. Using the data in Table III, a linear extrapolation of $\overline{M}_{w\,app}^{-1}$ *vs.* initial concentration to infinite dilution yields a $\overline{M}_{w\,app}$ value of 18,000 ± 1400, which is in excellent agreement with the estimate based upon amino acid composition.

Table II. Weight Average Molecular Weights for 95% Ethanol-Soluble Proteins from TE-77 Grain Sorghum

Initial protein concentration, mg/ml	$\overline{M}_w$ for nonredistributed proteins, g/mol
0.212	65,400
0.179	60,400
0.146	59,000
0.110	44,600

Table III. Weight Average Molecular Weight for a Single Component from TE-77 Prolamine

Initial protein concentration, mg/ml	$\overline{M}_w$ for nonredistributed component, g/mol
0.408	27,500
0.320	22,000
0.270	22,000
0.136	20,500

Finally the authors wish to call attention to observations made by Wu *et al.* (1970). These workers noted that the optical rotatory dispersion properties of sorghum prolamines do not change during the formation of gels in 60% v/v *tert*-butanol–water solvent. While the addition of GHCl to a 1.5 M concentration prevented gel formation, the optical properties changed only slightly. These workers noted that in 6 M GHCl solution the secondary structure of these proteins was changed markedly. In view of these observations and the results presented above, it appears that the proteins of sorghum prolamines can undergo very strong noncovalent associations which can persist in solution even in the presence of high concentrations of GHCl.

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