Proposed Nomenclature for the Alcohol-Soluble Proteins (Kafirins) of *Sorghum bicolor* (L. Moench) Based on Molecular Weight, Solubility, and Structure[†]

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Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and differential solubility were used to demonstrate similarities between maize zeins and sorghum kafirins. Polypeptides with similar apparent molecular weights (M_r) were found in the zein and kafirin fractions. Kafirins had two major bands at M_r 25 000 and 23 000 and four minor bands at M_r 28 000, 20 000, 18 000, and 16 000. Sorghum kafirins were separated into three overlapping groups on the basis of solubility differences: M_r 25 000 and 23 000 (soluble in 40–90% *tert*-butyl alcohol plus 2-ME); M_r 20 000, 18 000, and 16 000 (soluble in 10–60% *tert*-butyl alcohol plus 2-ME); and M_r 28 000 (soluble in 10–80% *tert*-butyl alcohol plus 2-ME). To identify structural similarities among the kafirin polypeptides and the zein polypeptides, Western blot analysis was carried out by using α-, β-, and γ-zein antiserum. Reactions were observed between α-zein antiserum and M_r 25 000 and 23 000 kafirin polypeptides, between β-zein antiserum and M_r 20 000 karifin polypeptide, and between γ-zein antiserum and M_r 28 000 kafirin polypeptide. A nomenclature for sorghum kafirins is proposed based on similarities in molecular weight, solubility, and structure to zeins.

Kafirin, the alcohol-soluble prolamin protein fraction in sorghum endosperm, makes up about 50% of the grain protein (Paulis and Wall, 1979). Landry and Moureaux (1970) devised an extraction scheme that divides the prolamins into two classes: true prolamins, extracted with 70% 2-propanol, and cross-linked prolamins, extracted with 70% 2-propanol plus 0.6% 2-mercaptoethanol (2-ME). Jones and Beckwith (1970) found that *tert*-butyl alcohol was a superior solvent to 2-propanol for extracting sorghum prolamins (kafirins). According to gel electrophoretic banding patterns and amino acid composition, there were no differences between the true kafirins and the cross-linked kafirin fractions (Paulis and Wall, 1979; Taylor et al., 1984).

A number of workers have used gel electrophoresis to separate the sorghum kafirins (Sastry and Virupaksha, 1967, 1969; Jones and Beckwith, 1970; Beckwith and Jones, 1972; Paulis and Wall, 1979; Taylor et al., 1984; Evans et al., 1987; Krishnan et al., 1989). While Sastry and Virupaksha (1967) were able to separate kafirins from Indian sorghum into seven or eight components, Krishnan et al. (1989) were the first to assign molecular weights to the kafirin protein bands. They observed two major kafirin polypeptides with M_r 27 000 and 25 000 and two minor polypeptides with M_r 18 000 and 12 000. Other than the assignment of molecular weights, little else has been done to classify the kafirin polypeptides.

In contrast to sorghum, a vast amount of work has been published on identification, characterization, and isolation of the alcohol-soluble zein components in maize. When separated by SDS–PAGE maize storage proteins are resolved into polypeptides of M_r 27 000, 22 000, 19 000, 16 000, 14 000, and 10 000 (Larkins et al., 1984). Wilson (1985b) proposed a nomenclature system for the zein polypeptides based on the apparent molecular weight when analyzed by SDS–PAGE. It is as follows: reduced soluble protein (M_r 27 000), A-zein (approximate M_r 24 000), B-zein (approximate M_r 22 000), C-zein (M_r 15 000–18 000), and D-zein (M_r 9000–10 000). More recently, Esen (1987) proposed a nomenclature system for the zeins based on solubility: α-zein (soluble in 50–95% 2-propanol), β-zein (soluble in 30–85% 2-propanol), and γ-zein (soluble in 0–80% 2-propanol containing a reducing agent). The α-zein polypeptides correspond to the M_r 21 000–25 000 and 19 000 bands. These comprise 75–85% of the total zein depending on the genotype and consist of a heterogeneous mixture of proteins. They are composed of four to five bands, and each of these can be separated into differently charged species by isoelectric focusing (Hastings et al., 1984; Wilson, 1985b). The β-zeins correspond to the M_r 17 000 and 18 000 polypeptides (Esen, 1987) or the M_r 16 000 and 14 000 bands (Lending et al., 1988). They make up 10–15% of the total zein. Each molecular weight class is made up of one or two polypeptides. The β-zeins are higher in sulfur-containing amino acids (Esen et al., 1985; Pedersen et al., 1986) and include the high-methionine zein component reported by Esen et al. (1985) and Gianazza et al. (1977).

γ-Zein has an M_r of 27 000 and makes up 5–10% of the total zein. It has been reported to be soluble in aqueous solvents plus reducing agent. There is some disagreement as to whether it is actually a prolamin (Esen, 1987). More recent research has identified the M_r 18 000 zein as a truncated version of the γ-zein (M_r 27 000) and has been termed γ-zein$_2$ (Esen, 1990). There is another zein component with M_r 10 000 that contains a high proportion of sulfur amino acids. It differs in solubility from β-zein (Esen, 1986). Esen (1987) termed it a zein on the basis of its solubility in 50–95% 2-propanol and suggested that the M_r 10 000 protein be named δ-zein.

While isolation and characterization of sorghum kafirins have not advanced as far as for maize zeins, many similarities have been found between proteins of the two grains. Therefore, classification schemes for sorghum that correspond to those in maize are frequently used. Sastry and Virupaksha (1969) identified a group of kafirins soluble

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in 95% ethanol that are analogous to the α-zeins in maize. These workers referred to these sorghum polypeptides as α-kafirins. The remaining kafirins that are insoluble in 95% ethanol are termed β-kafirins. Evans et al. (1987) and Taylor et al. (1989) have identified and isolated a protein of M_r 28 000–30 000 which has been referred to as reduced-soluble protein (RSP) corresponding to maize RSP (Wilson, 1983). Similar isolation of the M_r 28 000 kafirin was carried out by Watterson et al. (1990).
To bring clarity to the nomenclature of sorghum kafirins, this paper identifies the SDS–PAGE banding patterns of the alcohol-soluble sorghum proteins and presents a nomenclature system for these proteins. SDS–PAGE, solubility, and immunological techniques were used to identify similarities between maize zeins and sorghum kafirins.

MATERIALS AND METHODS

Maize and Sorghum Extract Preparations. A pure line variety of sorghum SC283-14 grown at the Purdue University Agronomy Farm during the 1986 crop season was used throughout this study. The sorghum seed were degermed and decorticated with a Strong-Scott barley pearler prior to pulverization. Commercial maize flaking grits containing a mixture of genotypes and decorticated sorghum were ground in a Tecator Cyclone mill through a 0.4-mm screen. Two-gram quantities of flour were placed in a 50-mL centrifuge tube for extractions. Maize and sorghum flours were extracted with 20 mL of 70% 2-propanol (v/v) plus 5% 2-ME (v/v) and 20 mL of 60% *tert*-butyl alcohol (v/v) plus 5% 2-ME (v/v), respectively, for 5 h at 37 °C on a Labquake rocking plate shaker. Extracts were centrifuged for 5 min at 5000*g*, and the supernatants were removed. Fresh protein extracts were prepared daily. Protein concentration of extracts were quantitated by the Kjeldahl method (AACC Method 46-11) using $CuSO_4$–TiO_2 as the catalyst.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). SDS–PAGE was carried out on a LKB horizontal Multiphor II electrophoresis system on gel slabs (200 × 260 × 2 mm). Two types of running gels were used for analysis. The first type was a linear 8–18% polyacrylamide gradient in 0.3 M Tris buffer and 0.1% SDS (w/v, pH 8.8) with a stacking gel containing 0.125 M Tris buffer (pH 6.8), 4% polyacrylamide (w/v), and 0.1% SDS (w/v). The second type was a linear 10–15% polyacrylamide gradient in 0.4 M Tris buffer and 0.1% SDS (w/v, pH 8.8) with 6 M urea. The stacking gel contained 0.125 M Tris buffer (pH 6.8), 4% polyacrylamide (w/v), 6 M urea, and 0.1% SDS (w/v). Gels were polymerized with tetramethylethylenediamine and ammonium persulfate. Tank buffer was 0.025 M Tris and 0.19 M glycine containing 0.1% SDS (w/v). Protein extracts were diluted in sample buffer [0.063 M Tris, pH 6.8, 2.5% SDS (w/v), 0.48 M glycine, and 2.5% 2-ME (v/v) in 0.05% bromophenol blue (w/v)] to a concentration of 5 μg/μL, heated at 100 °C for 3 min, and immediately cooled with ice. One hundred micrograms of protein was loaded into each well. Standard reference proteins (Dalton Mark VII-L) from Sigma (St. Louis, MO) of low molecular weight [α-lactalbumin (14 200); soybean trypsin inhibitor (20 100); PMSF-treated trypsinogen (24 000); carbonic anhydrase (29 000); glyceraldehyde-3-phosphate dehydrogenase (36 000); egg albumin (45 000); bovine albumin (66 000)] were used. Electrophoresis was carried out at 200 V for 14 h. Proteins were stained with 0.05% Coomassie Brilliant Blue R-250 (w/v) in 10% TCA (w/v) and 40% methanol (v/v). The gels were destained with 10% TCA (Wilson, 1986). Molecular weights were determined from standard curves obtained by plotting $\log_{10}$ molecular weight against relative mobility.

Differential Solubility Procedure. One-gram quantities of sorghum flour were placed in 10 50-mL centrifuge tubes for extraction. Samples were extracted with 10 mL of 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90% *tert*-butyl alcohol (v/v) plus 1% 2-ME (v/v) on a Labquake rocking plate for 3 h at room temperature. Extracts were centrifuged for 5 min at 5000*g*, and supernatants were removed and freeze-dried. Freeze-dried protein was dissolved in sample buffer to give a final protein concentration of 7 μg/μL. Two hundred micrograms of protein was loaded on the linear 10–15% polyacrylamide gradient gel with urea.

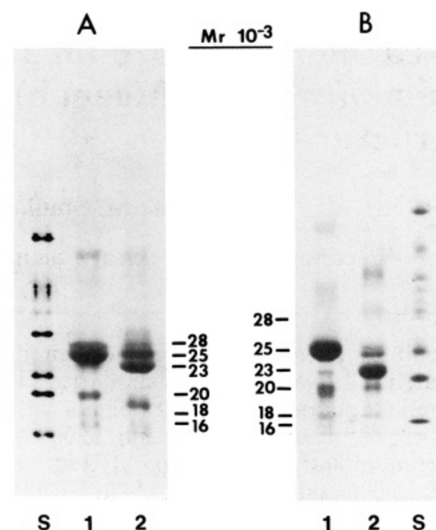

Figure 1. SDS–PAGE profiles of total kafirin (lane 1), total zein (lane 2), and molecular weight standards (lane S) using (A) a linear 8–18% polyacrylamide gradient gel without urea and (B) a linear 10–15% polyacrylamide gel with urea.

Antibody Production. Antisera to α-, β-, and γ-zeins and a preimmune serum were obtained from Dr. B. A. Larkins' laboratory, University of Arizona (Lending et al., 1988). The α-zein (M_r 21 000–25 000 and 19 000), β-zein (M_r 18 000, 17 000, and 15 000), and γ-zein (M_r 27 000) types were isolated from maize meal according to the method of Esen (1986). Antiserum was prepared by injection into New Zealand White rabbits and prepared from blood by standard methods (Cooper, 1977).

Western Blot Analysis. Proteins were transferred from SDS–PAGE gels to nitrocellulose sheets (Towbin et al., 1979) by using a continuous buffer system [39 mM glycine, 48 mM Tris, 0.0375% SDS (w/v), and 20% methanol (v/v)] in a LKB Multiphor II Novablot cell. Duplicate transfers were carried out at 80 mA for 1 h. One nitrocellulose sheet was stained for total protein with 0.1% Amido Black 10B (w/v) in 10% acetic acid (v/v) and 45% methanol (v/v). The other was tested for reaction with rabbit antibodies to maize zeins. Nonspecific binding to nitrocellulose membranes was blocked by 3% nonfat dried milk in TBS (10 mM Tris, pH 7.5, 140 mM NaCl). Membranes were washed and incubated with either 1:1000 dilution of rabbit anti-α-zein serum, 1:100 dilution of rabbit anti-β-zein serum, 1:100 dilution of rabbit anti-γ-zein serum in buffer, or 1:100 dilution of preimmune serum. Bio-Rad (Richmond, CA) goat anti-rabbit horseradish peroxidase conjugate (GAR–HRP) was used as a secondary antibody at a 1:3000 dilution. Sites of antibody–protein association were visualized by reaction with hydrogen peroxide and 4-chloronaphthanol (Bio-Rad).

RESULTS

SDS–PAGE. A linear 8–18% polyacrylamide gradient gel without urea was used to compare banding patterns of zein and kafirin polypeptides extracted from maize and sorghum, respectively (Figure 1). This gel effectively separates the lower molecular weight polypeptides (M_r 23 000–16 000) where the bulk of the prolamin polypeptides exist. With our electrophoretic system, the zein polypeptides separated into seven bands with M_r 28 000, 25 000 doublet, 23 000, 18 000, 17 000, and 15 000. The major bands were M_r 25 000 (doublet) and M_r 23 000. The staining intensity of the M_r 23 000 band was greater than that of the M_r 25 000 band. There were three minor bands of M_r 18 000, 17 000, and 15 000, and an M_r 28 000 polypeptide which was previously described as M_r 27 000 (Esen et al., 1981).

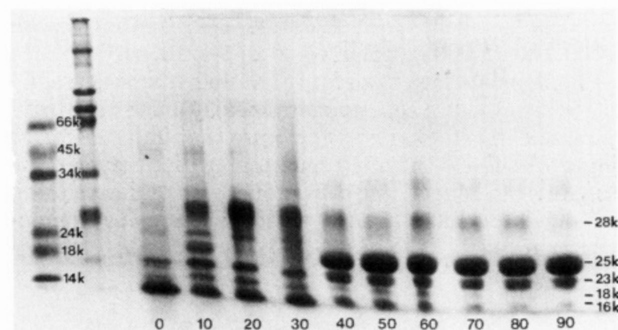

Figure 2. SDS-PAGE (linear 10-15% polyacrylamide gradient with urea) profile of kafirin differential solubility fractions. Number indicates the percent *tert*-butyl alcohol in extracting solvent.

The electrophoretic banding pattern of sorghum kafirin was similar to that of the maize zeins (Figure 1). It consisted of six bands with M_r 28 000, 25 000, 23 000, 20 000, 18 000, and 16 000. The major bands were M_r 25 000 and 23 000. The M_r 25 000 band was resolved into a doublet and is present in higher quantity than the minor M_r 23 000 band. This is the opposite of what was found with maize zeins. The lower molecular weight bands appeared as three independent bands. The M_r 28 000 kafirin polypeptide was similar to the M_r 27 000 zein in that it has a faint, broad staining pattern.

The linear 10-15% polyacrylamide gradient gel with urea showed a similar banding pattern to the gel without urea; however, differences in rates of migration were observed. On the gel with urea, the M_r 28 000 kafirin in particular migrated more slowly and appeared more diffuse. Other differences were the appearance of a number of lower molecular weight bands ($<M_r$ 18 000) in the urea gel that were not visible on the gel without urea. Molecular weight calculations cannot be made due to the addition of urea to the gel. However, the results indicate that there are perhaps more low molecular weight kafirins than we have identified.

Differential Solubility. A linear 10-15% polyacrylamide gradient gel with urea was used to separate the polypeptides in the differential solubility fractions (Figure 2). Differences in protein solubility were determined by observing changes in the banding profiles as percent alcohol in the solvent increased. The M_r 25 000 and 23 000 kafirin bands were extractable in 40-90% *tert*-butyl alcohol plus 2-ME, while the lower molecular weight kafirin bands (M_r 20 000, 18 000, and 16 000) were extractable in 10-60% *tert*-butyl alcohol plus 2-ME. The M_r 28 000 kafirin was extractable in 10-80% *tert*-butyl alcohol plus 2-ME.

Western Blots. To determine structural similarities between individual zeins and kafirin polypeptides, kafirins were tested for immunogenic reactivity with α-, β-, and γ-zein antisera. A linear 8-18% polyacrylamide gradient without urea was used to separate kafirin and zein protein extracts for analysis of α- and β-zein antiserum reaction since this gel effectively separates the lower molecular weight polypeptides. A 10-15% linear gradient gel with urea was used to separate kafirin and zein proteins for γ-zein antiserum reaction since this gel provides separation in the region between M_r 45 000 and 25 000. The latter gel effectively separates the α-zein (M_r 25 000 and 23 000) and γ-zein (M_r 27 000). Results of the Western blot analysis are shown in Figures 3 and 4. Antibodies produced to α-zeins (M_r 25 000 and 23 000) strongly reacted with the corresponding maize bands and reacted equally as strongly with the M_r 25 000 and 23 000 kafirin polypeptides (Figure 3).

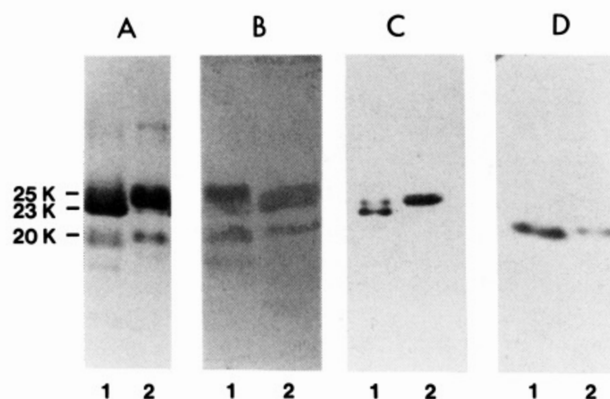

Figure 3. Immunospecificity of α- and β-zein antiserum. (A) SDS-PAGE (linear 8-18% polyacrylamide gradient without urea) profile of total zein (lane 1) and total kafirin (lane 2). (B) Amido Black stain of total zein (lane 1) and total kafirin (lane 2) extracts transferred from SDS-PAGE to nitrocellulose. (C) Western blot of total zein (lane 1) and total kafirin (lane 2) reacted with α-zein antiserum. (D) Western blot of total zein (lane 1) and total kafirin (lane 2) reacted with β-zein antiserum.

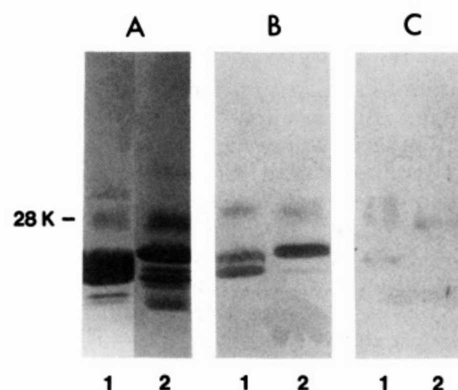

Figure 4. Immunospecificity of γ-zein antiserum. (A) SDS-PAGE (linear 10-15% polyacrylamide gradient with urea) profile of total zein (lane 1) and total kafirin (lane 2). (B) Indian ink stain of total zein (lane 1) and total kafirin (lane 2) extracts transferred from SDS-PAGE to nitrocellulose. (C) Western blot of total zein (lane 1) and total kafirin (lane 2) reacted with γ-zein antiserum.

The β-zein (M_r 18 000, 17 000, and 15 000) antibodies showed cross-reactivity with only the M_r 18 000 maize polypeptide and the M_r 20 000 kafirin polypeptide. Reactivity between the β-zein antiserum and the M_r 20 000 kafirin was also weaker than with the corresponding zein polypeptide. The lack of reactivity of the β-zein antiserum with either the M_r 17 000 or 15 000 zein bands or the M_r 18 000 and 16 000 kafirin bands may be due to the low protein concentration in these bands. γ-Zein antiserum reacted positively with the M_r 27 000 zein and the M_r 28 000 kafirin polypeptides.

DISCUSSION

Zein components were defined as α-, β-, and γ-types on the basis of solubility. α-Zeins (M_r 25 000 and 23 000) are extracted in 50-95% alcohol, β-zeins (M_r 18 000, 17 000, and 15 000) are extracted in 30-85% alcohol, and γ-zein (M_r 27 000) is extracted in 0-80% alcohol plus a reducing agent (Esen, 1987). Accordingly, the kafirin polypeptides extracted under conditions similar to those used for the corresponding zeins. M_r 25 000 and 23 000 kafirin polypeptides were electrophoretically similar to α-zein and also had similar extractability, requiring relatively high concentrations of alcohol to be solubilized (40-90%). The lower molecular weight kafirin polypeptides (M_r 20 000,

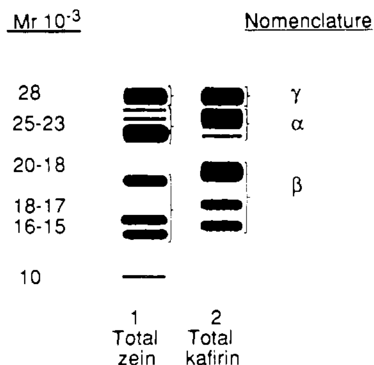

Figure 5. Diagrammatic representation of total zein (lane 1) and total kafirin (lane 2) polypeptides extracted with 70% alcohol and 2-ME and separated by SDS-PAGE.

18 000, and 16 000) were extracted only with lower amounts of alcohol (10–60%). This makes them similar to β-zeins, which are less alcohol soluble than the α-zein types. Finally, the extractability of the M_r 28 000 kafirin polypeptide was very similar to that of γ-zein. This protein was extracted within a wide range of alcohol concentrations (10–80%) in the presence of a reducing agent.

In addition to similarities in electrophoretic mobility and alcohol solubility, immunological analysis showed structural similarities between kafirin and zein polypeptides. Antiserum produced from α-, β-, and γ-zeins reacted with total kafirin extracts. Western blot analysis showed α-, β-, and γ-zein antisera to react with M_r 23 000–25 000, 20 000, and 28 000 kafirins, respectively. The γ-zein antiserum also reacted with a lower molecular weight band in maize. Similar cross-reaction was observed by Esen (1987), where γ-zein antiserum reacted with the M_r 18 000 band.

Such cross-reactivity between zeins and other prolamins is not unique to sorghum. Immunological studies have shown cross-reactivity among the prolamins from maize, wheat, and barley. Dierks-Ventling and Cozen (1982) produced antiserum to whole zeins and showed that it reacted with certain gliadin and hordein components. The reverse reaction, however, did not occur. While antiserum produced to hordien reacted with certain gliadin components, it did not react with zein. Recently, Esen (1988) used monoclonal antibodies to four α-zein components to show structural similarities among the individual α-zein polypeptides. Esen concluded that extensive cross-reactivity existed among the α-zein polypeptides and attributed it to shared epitopes. He also conjectured that differences in levels of cross-reactivity may be due to the number of epitopes two polypeptides have in common or the affinity of the antibody to some epitopes.

A number of nomenclature systems have been proposed for maize zeins but none to date for sorghum kafirins. We propose the following nomenclature for kafirin polypeptides which is based on an existing zein nomenclature and emphasizes the similarities in molecular weight, solubility, and structure between kafirins and zeins. Accordingly, the M_r 23 000 and 25 000 polypeptides are α-kafirins due to their solubility in 40–90% *tert*-butyl alcohol plus 2-ME and their cross-reactivity with the α-zein (Figure 5). The M_r 20 000 polypeptide is a β-kafirin due to its solubility in 10–60% *tert*-butyl alcohol plus 2-ME and cross-reactivity of the β-zein. The M_r 28 000 is a γ-kafirin due to its solubility in 10–80% *tert*-butyl alcohol plus 2-ME and its cross-reactivity with the γ-zein. While a positive reaction between β-zein antiserum and M_r 18 000 and 16 000 bands was not observed, they have a similar solubility to β-zein. We, therefore, suggest that the M_r 18 000 and 16 000 be referred to as β-kafirins.

A nomenclature system for sorghum kafirins is greatly needed to provide a common terminology among researchers. Use of a common nomenclature that imitates maize zeins and reflects the numerous similarities between the two grains will encourage consistency and understanding and alleviate what currently exists as confusing nomenclatures for zein.

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