Distribution and Electrophoretic Properties of Alcohol-Soluble Proteins in Normal and High-Lysine Sorghums

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ABSTRACT

Electrophoretic patterns and amino acid compositions were compared between alcohol-soluble proteins from normal and high-lysine sorghum grain meals. Sequential extraction of grain meals by a modified Osborne-Mendel scheme confirmed findings that the high-lysine sorghums contained more salt-soluble and alcohol-insoluble reduced glutelins and less alcohol-soluble proteins, kafirin, and alcohol-soluble reduced glutelin (ASRG) than did the normal sorghums. Kafirin and ASRG had similar amino acid compositions in each variety and differed only slightly between varieties. Kafirin and ASRG polypeptides migrated primarily as a prominent broad band with an apparent molecular weight of 22,000 during sodium dodecyl sulfate-polyacrylamide gel electrophoresis, but they were heterogeneous by polyacrylamide gel electrophoresis in 8*M* urea-aluminum lactate pH 3.1. Electrophoretic patterns of kafirins and ASRGs for near-isogenic normal and high-lysine genotypes were similar, but different, between unrelated varieties. These data suggest a close relationship between kafirins and ASRG proteins. Polyacrylamide gel electrophoresis is useful for characterizing, comparing, and differentiating sorghum alcohol-soluble proteins in genetic investigations.

Virupaksha and Sastry (1968) demonstrated that sorghum endosperms contained proteins corresponding to each of the Osborne-Mendel extraction fractions. Yields of total extracted proteins in six varieties range from 80.7 to 103.4%. Later, Jambunathan and Mertz (1973) fractionated sorghum proteins using the procedure of Landry and Moureaux (1970). This procedure solubilizes most of the nitrogenous compounds of sorghum and yields five fractions. The alcohol-soluble proteins of sorghum, kafirin, and alcohol-soluble reduced glutelin (ASRG) represent the largest fraction of proteins in the grain. Recent discoveries of high-lysine sorghums (Singh and Axtell 1973, Mohan and Axtell 1975) have prompted investigations of the proteins in those grains. Jambunathan et al (1975) demonstrated that the ratio of alcohol-soluble proteins to total protein is lower in high-lysine sorghum grain than in normal grain. This relationship is true of the zein and ASRG fractions in corn, which are similar to the alcohol-soluble proteins of sorghum in amino acid composition and solubility (Landry and Moureaux 1970, Paulis and Wall 1971, Sodek and Wilson 1971). The polypeptide compositions of these proteins in corn have been shown to exhibit much variation in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Gianazza et al 1976, Lee et al 1976, Misra et al 1976) and PAGE in 8*M* urea aluminum lactate pH 3.1 (Paulis and Wall 1977a). To establish if differences occur in distribution and gel electrophoretic patterns of protein from unrelated and near-isogenic sorghums, two high-lysine and two normal grains were examined. Since differences do occur, PAGE may be used as a method for comparing and differentiating alcohol-soluble proteins in genetic investigations of sorghums.

MATERIALS AND METHODS

Treatment of Grain

The high-lysine sorghum grains used in this study were P721 opaque (*o*), a chemically induced mutant (Mohan and Axtell 1975), and IS11758, an Ethiopian variety (Singh and Axtell 1973). Grains of two normals, P721+, an inbred used to prepare the P721 *o*, and TE77, a widely grown hybrid, were also investigated. The grains were ground rapidly in a Udy sample cyclone hammer mill through a screen with round 0.6-mm openings. Lipid was removed from the meals with *n*-butyl alcohol (Jones and Dimler 1962).

Mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.

Isolation of Protein

Jones and Beckwith (1970) developed the methods used to isolate albumin, globulin, and kafirin fractions from defatted grain sorghum meals. ASRGs were extracted next from the meal with 60% *t*-butanol containing 0.1*M* β-mercaptoethanol (ME) by a procedure similar to that used for kafirin extraction. Alcohol-insoluble reduced glutelins (AIRG) were extracted last from the meals with the borate pH 10, 0.6% ME, and 0.5% SDS buffer used in the Landry-Moureaux procedure (1970).

Analytic Methods

Aliquots of extracts or portions of weighed, dried materials were assayed for nitrogen by a semimicro-Kjeldahl method. Crude protein (N × 6.25) was given on an as-is basis.

Samples for amino acid analysis were hydrolyzed by refluxing in 6*N* HCl (2 ml/mg sample) for 24 hr and analyzed with a Beckman amino acid analyzer following a previously described procedure for quantitation (Paulis and Wall 1977a). All amino acids were corrected to 97% recovery of nitrogen for comparison between samples.

SDS-PAGE of the polypeptides from kafirin and ASRG preparations was done in 10% gels in SDS-borate, pH 8.9, whereas PAGE was conducted in 5% gels in 8*M* urea-aluminum lactate, pH 3.1 (Paulis and Wall 1977a). The proteins for PAGE were reduced with ME and alkylated with acrylonitrile (Paulis and Wall 1977a).

RESULTS AND DISCUSSION

Data in Table I demonstrate the differences in protein distribution between high-lysine (P721 *o* and IS11758) and normal sorghums (P721+ and TE77). The water, saline, and AIRG proteins are present in larger amounts in the two high-lysine varieties than in the normals. The amounts of ASRG in normal and high-lysine grains are similar. The amount of kafirin is much higher in the normal than in the high-lysine sorghum grains. Contents of kafirin and ASRG in the normals are similar, whereas much more ASRG than kafirin appears in the high-lysine varieties. The total of

kafirins and ASRGs averages 54.4% of the grain protein in the normals as compared with 33.4% in the high-lysine sorghums. Since the water, saline, and AIRG proteins have a high content of lysine and the alcohol-soluble proteins (kafirin and ASRG) are low in this amino acid (Jambunathan and Mertz 1972), the relative changes in the five protein fractions account for the increase in the lysine content in the high-lysine sorghum kernel. These yields of protein fractions from normal and high-lysine sorghums are consistent with those reported by Jambunathan et al (1975).

The amino acid compositions of individual alcohol-soluble proteins are listed in Table II. The kafirins and ASRG of different varieties differ only slightly in amino acid content. The kafirins and ASRG for each variety have similar amino acid compositions. This similarity between the ASRGs and kafirins suggests that ASRG was derived from disulfide cross-linked kafirin solubilized by adding ME to the butanol solvent. Sorghum ASRG does not appear to be a unique protein fraction as is ASRG found in corn (Paulis and Wall 1971, 1977b; Sodek and Wilson 1971).

The SDS-PAGE patterns of the kafirins of all the varieties have mainly one broad band migrating in the 22,000 dalton region (Fig. 1). The normal kafirin bands (patterns 1 and 3) seem to migrate slightly faster than the high-lysine ones (patterns 2 and 4). This wide kafirin band at 22,000 daltons probably consists of at least two components that have not been resolved. The mobility of this kafirin band is similar to that of the prominent band observed during SDS-PAGE of ASRG from all four varieties. A faint band also appears around 12,000 daltons in all the ASRGs and kafirins. The ASRG of IS11758 (pattern 6) has a faint band around 50,000 daltons.

PAGE patterns in aluminum lactate-8*M* urea pH 3.1 buffer (Fig. 2) of the reduced and alkylated kafirins from the four grains exhibit similarities as well as marked significant differences. Pattern 1 of the hybrid TE77 contains two bands in region I not seen in the inbred varieties. In this region, the fastest moving band is the most intense in P721+ (pattern 3). In regions II and III, P721+ and P721 *o* are the same, containing four bands, some of slightly different intensities. Patterns 1 and 2 of kafirins from TE77 and IS11758 contain only three bands in region II. One TE77 band has a different mobility than those of the other kafirins. The reduced and alkylated ASRGs exhibit PAGE patterns almost identical to the corresponding patterns of kafirins.

The results of SDS-PAGE and PAGE of kafirin proteins in aluminum lactate-8*M* urea buffer differ greatly. During SDS-PAGE (Fig. 1), all the kafirins and ASRGs behave similarly, migrating mostly as one broad prominent band around 22,000 daltons. Each of these broad SDS-PAGE bands must consist of

TABLE I
Protein Distribution in Whole Kernels of Normal and High-Lysine Sorghums

		Normal		High Lysine	
		P721+	TE77	IS11758	P721 *o*
		% Protein in Meal (As Is)			
		13.1	10.7	15.0	15.4
Fraction	Proteins	% of Total Nitrogen Extracted			
Water	Albumins and nonprotein nitrogen	8.0	6.2	11.4	9.7
1% NaCl	Globulins	5.2	3.7	7.9	7.3
60% *t*-Butanol	Kafirins	26.2	29.9	7.6	13.0
60% *t*-Butanol-0.1*M* ME[a]	Alcohol-soluble reduced glutelins	28.3	24.4	25.3	20.9
Borate pH 10 + 0.6% ME + 0.5% SDS[b]	Alcohol-insoluble reduced glutelins	25.5	25.6	37.8	36.5
Total nitrogen extracted (%)		93.2	89.8	90.0	87.4

[a]ME = mercaptoethanol.
[b]SDS = sodium dodecyl sulfate.

TABLE II
Amino Acid Compositions of Alcohol-Soluble Proteins of Normal and High-Lysine Sorghums (g/100 g Protein)

	Kafirins				Alcohol-Soluble Reduced Glutelins			
Amino Acids	P721+	P721 *o*	IS11758	TE77	P721+	P721 *o*	IS11758	TE77
Lysine	0.1	0.2	0.1	0.1	0	0.1	0	0.2
Histidine	0.7	0.7	1.0	1.1	1.0	1.3	0.8	1.3
Ammonia	4.4	5.9	3.9	4.5	4.5	4.3	4.1	4.4
Arginine	1.8	1.7	1.6	0.9	1.0	2.1	1.5	1.1
Aspartic acid	6.7	5.8	6.6	6.2	5.3	5.6	6.0	5.4
Threonine	2.7	2.3	2.7	2.4	2.6	2.5	2.6	2.4
Serine	4.1	3.5	4.2	4.2	4.5	4.1	4.6	4.5
Glutamic acid	23.8	20.4	24.2	23.2	22.3	22.5	23.4	22.7
Proline	10.3	9.2	10.5	10.8	11.2	10.4	11.2	11.0
Glycine	1.4	1.5	1.2	1.4	1.7	1.5	1.5	1.5
Alanine	12.1	10.6	12.9	12.0	11.9	11.4	12.3	12.0
Valine	5.6	4.7	5.1	4.5	4.5	4.5	4.6	4.4
Methionine	0.6	1.3	0.8	1.2	1.4	1.8	0.8	1.4
Isoleucine	4.2	3.8	4.4	4.4	4.4	4.1	4.6	4.3
Leucine	18.1	15.6	18.9	18.6	18.3	17.5	19.4	18.1
Tyrosine	4.7	4.2	5.1	4.9	4.9	4.8	4.9	4.6
Phenylalanine	5.8	5.2	6.3	6.2	6.2	5.8	6.9	6.2

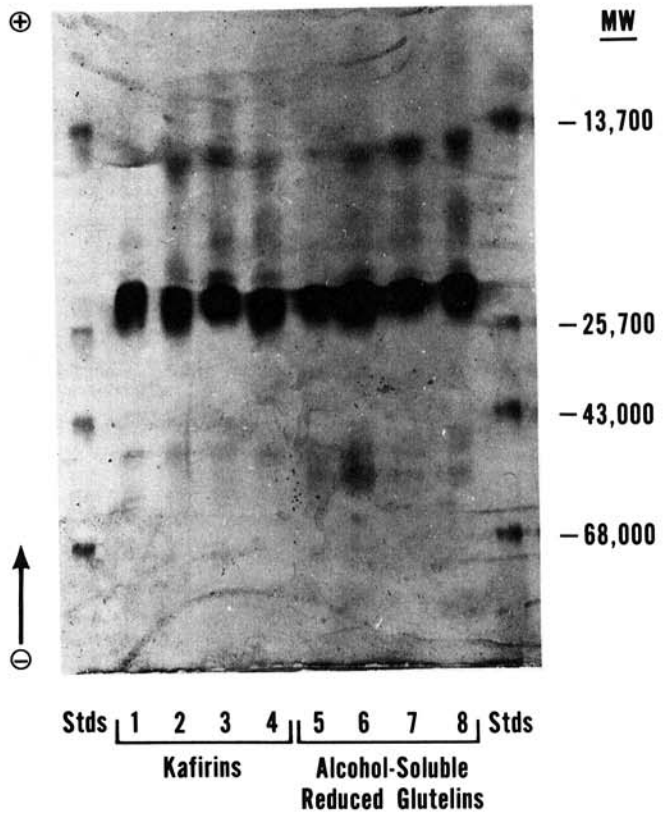

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of kafirins and alcohol-soluble reduced glutelins from normal and high-lysine sorghum. Standards: molecular weight (MW) calibration mixture; 1 and 5, TE77; 2 and 6, IS11758; 3 and 7, P721+; 4 and 8, P721 *o*. Origin at bottom.

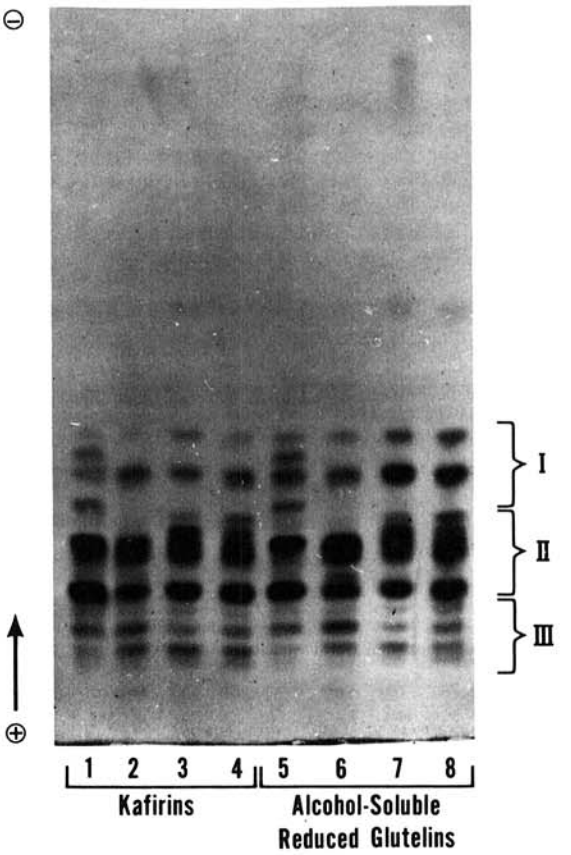

Fig. 2. Polyacrylamide gel electrophoresis patterns of kafirins and alcohol-soluble reduced glutelins from normal and high-lysine sorghums. 1 and 5, TE77; 2 and 6, IS11758; 3 and 7, P721+; 4 and 8, P721 *o*. Origin at bottom.

several polypeptides varying in charge and amino acid content, since a much greater number of components is evidenced in the PAGE patterns (Fig. 2). Although the SDS-PAGE patterns of the kafirins and ASRGs of the two normal grains show no major differences, their PAGE patterns do. The finding of qualitative similarity in PAGE patterns of the kafirins of normal and high-lysine P721 is of interest, since in these near-isogenic grains, only one gene has been changed. Some quantitative differences occur in the PAGE bands of kafirins from normal and high-lysine P721 grains.

Like the ASRG in corn, the sorghum ASRG fraction also contains lower molecular weight components as observed by SDS-PAGE (Fig. 1). A 12,000-mol wt component may be analogous to the fast-moving high-methionine polypeptides in corn ASRG (Gianazza et al 1977; Paulis and Wall 1971, 1977b). Similar low molecular weight polypeptides, however, are present in the kafirin preparation obtained by extracting with 60% *t*-butanol in the absence of ME. Zein components in corn do not show analogous low molecular weight constituents (Paulis and Wall 1977a). Since the PAGE and SDS-PAGE patterns of kafirins and ASRGs of the same sorghum variety appear qualitatively identical and their amino acid contents are similar, the constituent polypeptides are probably the same. The ASRGs in corn probably have an independent genetic origin, since they are different from zein (Gianazza et al 1977, Paulis and Wall 1977b).

The differences between varieties in PAGE patterns of the kafirins or ASRGs indicate that this technique might be used as a tool in following genetic changes during breeding. Since both kafirin and ASRG of the same variety gave similar patterns, examining each fraction by PAGE would not be advantageous. When samples are limited, an extraction with 60% *t*-butanol plus 0.1*M* ME, which removes both kafirins and ASRG, should be sufficient for PAGE examination. This extraction has been used to differentiate normal and high-lysine sorghum grains based on yield of extracted protein (Paulis and Wall 1975).

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