Electrophoretic and Developmental Characterization of Oat (*Avena sativa* L.) Globulins in Cultivars of Different Protein Content[1]

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ABSTRACT

Groat protein concentration in mature seeds from nine oat (*Avena sativa* L.) cultivars ranged from 11.9 to 19.1%. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the oat globulins under nonreducing conditions showed one major group of proteins of molecular weights 52,000–70,000. In the presence of a reducing agent, the majority of these proteins separated into two subunit groups: the α-subunit group (33,000–43,000 mol wt) and the β-subunit group (20,000–25,000 mol wt). Isoelectric focusing of the reduced and alkylated globulins showed two definite groups of proteins—one acidic (pH 5.5–7.5) and one more basic (pH 9.0–10.0)—that corresponded to the α- and β-subunit proteins, respectively. These findings were consistent for all the cultivars examined. The globulin banding pattern changed throughout seed maturation. During the first two weeks after anthesis, the lower molecular weight constituents of the α (33,000 and 35,000 mol wt)- and β (20,000 mol wt)-subunit groups were predominant. From the third week onward, synthesis of the higher molecular weight proteins in both groups prevailed, giving rise to the typical reduced-globulin pattern.

Because cereals provide much of the world's protein supply (about 50%) but have proteins of relatively poor quality, efforts are being made to improve both the quantity and the quality of the proteins found in cereals. The oat grain deserves special attention because it contains protein of greater nutritional value than other cereals (Howe et al 1965, Maruyama et al 1975, Youngs et al 1973). Unlike the storage proteins of major crops such as wheat, corn, barley, and rye, in which alcohol-soluble prolamins are the major fraction (Mossé 1968), the main fraction in oat consists of globulins (Peterson and Smith 1976). Although globulins are nutritionally superior to the low-lysine prolamins (Draper 1973), oat globulins have received less attention than the major fractions of other seed crops.

Avena has a particularly wide range in protein content, which makes it an interesting choice for genetic manipulation. Estimates of the grain content in oat generally range from 12.4 to 25.4% (Clamot 1979, Robbins et al 1971), but values as high as 35.2% have been reported (Frey et al 1975). Very little is known about how this significant variation may affect the quality of the different seed protein fractions. Robbins et al (1971) examined 289 samples of oat groats varying in protein content from 12.4 to 24.4% and found significant variations in the amino acid profiles of many cultivars, but a good amino acid balance was preserved as protein content increased.

We studied the relationship between total seed protein content and the molecular weight and charge distribution of globulins from nine oat cultivars of different protein content. Preliminary studies of globulin accumulation during seed development are also reported.

MATERIALS AND METHODS

Plant Material

Oat cultivars were provided by V. Burrows, Ottawa Research Station, Agriculture Canada. They consisted of the high-nitrogen cultivars Hinoat and Dal, the commonly cultivated Sentinel, Elgin, and Harmon, high-yielding OA-424-1, low-yielding OT-213, and two low-nitrogen cultivars, OA-269 and Donald. Each cultivar was grown in triplicate standardized four-row plots (3 m long, 0.3 m apart) in 1980. The land had been summer-fallowed the previous year, and 75 kg/ha of seeds were sown. Nitrogen was applied at a rate of 33.6 kg/ha in a 10:10:10 (NPK) fertilizer mixture. The two center rows of each plot were harvested and air-dried to an equilibrium moisture content of 9–12%. The cultivar Elgin was used for the developmental study; the different growth stages were collected at six weekly intervals following anthesis. At harvest 3 the endosperm was milky, whereas at harvest 6 the seeds were mature and dry.

Nitrogen Determination

Grains were manually dehulled. Groats were milled in a regular electric coffee grinder to obtain a fine meal. Six samples from each cultivar were dried overnight at 105°C and analyzed by the standard micro-Kjeldahl method (AOAC 1980). The protein conversion factor used was 6.25 (Peterson 1976).

Protein Extraction

Globulins were extracted using a modification of the method employed by Peterson (1978). Triplicate samples (3.0 g) were stirred for 3 hr at 28°C with 200 ml of 1*M* NaCl, 0.05*M* *tris* (hydroxymethyl)aminomethane (Tris) at pH 8.5. The slurry was centrifuged at 15,000 × *g* for 30 min at 4°C. The supernatant was dialyzed against running water for 48 hr to precipitate the globulins. This was followed by centrifugation at 22,000 × *g* for 45 min at 4°C (except for harvest 1, when all the salt-soluble proteins were used for analysis because of their low protein content). The pellet (globulin) was freeze-dried.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE system was a variant of that used by Laemmli (1970). The resolving gel contained 11% acrylamide, 0.17% *N,N'*-methylene bisacrylamide (bis), 0.1% SDS, and 4*M* urea in 0.375*M* Tris/HCl buffer, pH 8.8. The stacking gel was composed of 3.8% acrylamide, 0.63% bis, 4*M* urea and 0.1% SDS in 0.125*M* Tris/HCl buffer at pH 6.8. The running buffer was a 3.0% Tris, 14.4% glycine, and 0.1% SDS solution. Freeze-dried protein samples (10 mg/ml) were dissolved in a sample buffer containing 8*M* urea, 1.0% SDS, 0.0 or 1.0% 2-mercaptoethanol (ME) and 0.054*M* Tris, pH 6.8. Samples were heated in boiling water for 3 min before analysis. Electrophoresis was conducted overnight at 60 V constant voltage on a vertical Protean double-slab electrophoresis cell (Bio-Rad). Gels were stained with 0.2% Coomassie Brilliant Blue R-250 in acetic acid-methanol-water (10:25:65, v/v) for 6 hr and destained with the acetic acid-methanol-water solution.

Protein Alkylation

Sample alkylation was performed according to Shewry et al (1978). Globulin samples (at a ratio of 10 mg/ml) were stirred overnight at 4°C in a solution of 8*M* urea, 0.01*M* KCl, and 1.0% ME in 0.133*M* Tris/nitrate buffer at pH 7.5. The reduced samples were then alkylated for 2 hr at 20°C by adding 1.5% (v/v) 4-

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vinylpyridine. The reaction was stopped by the addition of glacial acetic acid, to pH 3.0. The solution was dialyzed overnight and the precipitated globulins freeze-dried.

Isoelectric Focusing (IEF)

The IEF gels (23.0 × 11.5 × 0.1 cm) consisted of 5.0% acrylamide, 0.2% bis, 6*M* deionized urea, and 5.0% pH 3–10 ampholyte (Pharmacia). Samples were dissolved in sample buffer containing 10 m*M* glycine, 6*M* urea (pH 8.0 adjusted with Tris) at a concentration of 10 mg/ml. The gel was prefocused at 10 W constant power for 20 min on a Pharmacia FBE-3000 flatbed apparatus. Aliquots (25 μl) were resolved for 2 hr at 15 W. Resolution was improved by adding the globulin samples at the anodic end. The pH gradient was measured using pI standards and a surface pH electrode (20° C). The proteins were fixed in 10% TCA for 2 hr, and the gel was washed overnight with running water. The gel was then shaken for 3 hr in 0.05% Coomassie Brilliant Blue G in acetic acid-methanol-water (10:25:65, v/v) and destained as previously described.

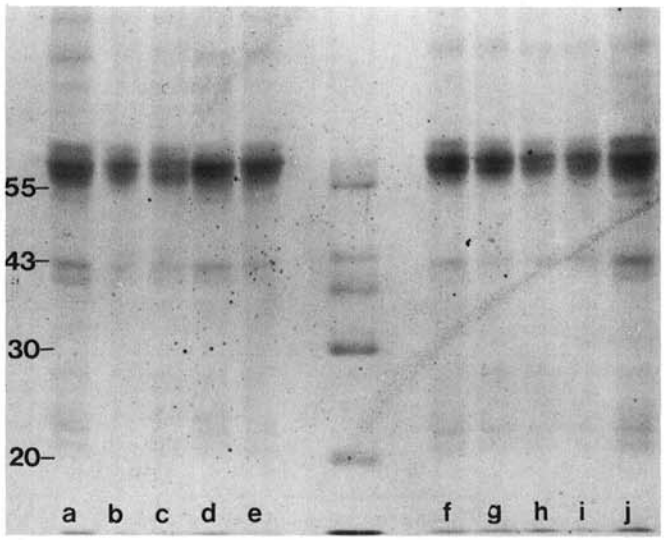

Fig. 1. Sodium dodecyl sulfate electrophoresis (without mercaptoethanol) of oat globulin fractions in cultivars: a, Hinoat; b, Dal; c, Sentinel; d, Elgin; e, Harmon; f, OA-424-1; g, OT-213; h, Donald; i, OA-269; and j, Hinoat. Molecular weight standards = bovine serum albumin (55,000 [Matta et al 1981]), ovalbumin (43,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (20,000).

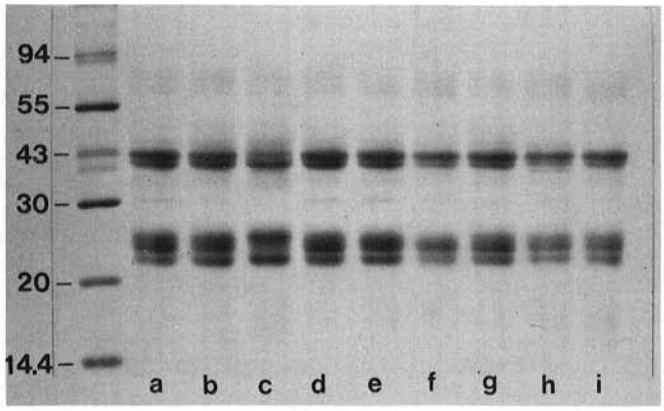

Fig. 2. Sodium dodecyl sulfate electrophoresis (with mercaptoethanol) of oat globulin fractions in cultivars: a, Hinoat; b, Dal; c, Sentinel; d, Elgin; e, Harmon; f, OA-269; g, OA-424-1; h, OT-213; and i, Donald. Molecular weight standards = phosphorylase b (94,000), bovine serum albumin (55,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000), and lactalbumin (14,400).

RESULTS AND DISCUSSION

SDS-PAGE Analysis of Oat Globulins

The groat protein concentrations of the nine cultivars were: 19.1 ± 0.3% (Hinoat), 18.1 ± 0.7% (Dal), 17.9 ± 0.8% (Sentinel), 17.0 ± 0.5% (Elgin), 15.6 ± 0.4% (Harmon), 15.6 ± 0.4% (OA-269), 14.8 ± 0.2% (OA-424-1), 14.5 ± 1.0% (OT-213), and 11.9 ± 0.1% (Donald). Each nitrogen determination is a mean of at least six values. Qualitative differences within the globulin fraction may distinguish the high- and low-protein cultivars. Globulin patterns obtained by SDS-PAGE under nonreducing conditions varied slightly (Fig. 1). All the cultivars showed a major group of proteins with molecular weights ranging from 52,000 to 70,000. Two major bands were consistently within this group, and differences among cultivars were confined to the relatively less important bands. Some minor constituents were also found in the higher molecular weight region and at approximately 36,000–40,000 and 20,000–25,000. Changes in the globulin patterns apparently were unrelated to variations in total protein content.

Reducing conditions altered the globulin electrophoretograms dramatically (Fig. 2). The nine globulin extracts showed a banding pattern similar to the oat globulin gel profile for the cultivar Froker (Peterson 1978), in which the majority of the globulin fraction consisted essentially of two subunits resolved by SDS-PAGE. These had molecular weights of 31,700 and 21,700, and are referred to as the α and β subunits, respectively (Peterson and Brinegar 1984). However, our results differed from this simple arrangement. Within the nine cultivars, the α subunit was generally comprised of two major polypeptides and up to four minor ones ranging in molecular weight from 33,000 to 43,000, whereas the β subunit involved up to four polypeptides between 20,000 and 25,000, of which three usually appeared as major bands. Hence, these proteins should perhaps more appropriately be referred to as the α- and β-subunit groups.

Considerable differences were noted in both the α- and β-subunit groups among the cultivars examined. A comparison between Hinoat and Sentinel illustrates this point clearly (Fig. 2). The presence or absence of protein bands as well as their differing relative intensities may be indicative of variations in the genetic regulation of the biosynthesis of oat globulins. Nonetheless, there was no corresponding increase or decrease of particular proteins with changes in total seed protein concentration within the nine cultivars studied.

The behavior of the oat globulins upon reduction implies that most proteins of the 52,000–70,000 family of globulins result from the association of the various components of the α- and β-subunit groups via disulfide linkages. The minor constituents at 36,000–40,000 and 20,000–25,000 seen in Fig. 1 are, therefore, probably globulins whose disulfide linkages were broken during sample boiling before electrophoresis. A few bands in the 52,000–70,000 molecular weight region persisted after reduction (Fig. 2); these may correspond to residual unreduced globulins, or they may be different proteins without disulfide linkage. Recent in vitro studies on oat globulin biosynthesis indicated that a 58,000 molecular weight precursor was post-translationally cleaved to yield the α and β subunits (Matlashewski et al 1982). The possibility of such an event was suggested by Luthe and Peterson (1977). Our results agree with these findings and may further indicate that a family of such "proglobulins" are synthesized, bringing about the various components of the α- and β-subunit groups. This situation is reminiscent of the legume globulins. For example, *Pisum sativum* legumin (Croy et al 1979), *Glycine max* glycinin (Staswick et al 1981), and *Vicia faba* legumin (Matta et al 1981) were all shown to involve disulfide-linked large acidic subunits and smaller basic counterparts. These have molecular weights very similar to the oat α- and β-subunit proteins respectively.

Isoelectric Focusing of Oat Globulins

Isoelectric focusing (IEF) was performed to investigate whether the oat globulins showed acidic and basic proteins analogous to legume globulins. Figure 3 shows the reduced and alkylated globulins of the nine cultivars separated on a pH 3–10 gradient.

Alkylation did not affect the number of bands obtained but increased the sharpness of resolution. Two definite groups of proteins were evident: one more acidic (pH 5.5–7.5) and the other consisting of basic polypeptides (pH 9.0–10.0). The acidic group contained the majority of the bands, although there were at least four bands in the basic group. Peterson (1978) reported that the α subunit contained significantly fewer basic amino acids than the β subunit; therefore, the α- and β-subunit groups probably correspond to the acidic and basic proteins, respectively. Analysis of the isolated subunit groups or two-dimensional IEF-SDS PAGE would provide further evidence. Oat would thus appear to resemble legumes not only in having a high proportion of globulins, but also in exhibiting an analogous mode of disulfide association of these proteins.

The higher resolution of IEF also demonstrated the high level of charge heterogeneity of the oat globulin fraction, which was not apparent upon SDS-PAGE analysis. Such intensive microheterogeneity for seed storage proteins has been documented, for example in wheat (Kasarda et al 1976), corn (Righetti et al 1977), barley (Shewry et al 1978), and broad bean (Matta et al 1981). These have been considered products of clusters of duplicated genes that diverged throughout evolution (Bietz et al 1977, Kasarda et al 1976, Thomson and Doll 1979). The same may apply to oat globulins. In view of the large number of bands and the difference in total protein content, the nine cultivars displayed similar IEF patterns. This would not support a random or artefactual origin of this heterogeneity and would favor a significant degree of conservatism within these proteins. Indeed, recent evidence on cereal prolamins seems to support significant conservatism in the form of homologous amino acid sequences preserved within a single protein (Geraghty et al 1981), within a prolamin fraction (Bietz et al 1979), or even among prolamins of different cereals (Shewry et al 1980). Mutational changes would thus seem to be limited to particular portions of the molecules. Such conservative restraints might explain the smaller number of bands found in the β-subunit group.

Appearance of Globulins During Seed Development

Recent studies showed that the N percentage of different oat cultivars varied significantly at all stages of seed development (Welch et al 1980).[4] In 1976, Peterson and Smith reported a marked increase in globulin synthesis within the first 16 days following anthesis. The proportion of this fraction rose from 28 to 55% during seed development. An equivalent study of the qualitative aspects of oat globulin accumulation is still lacking.

Globulins extracted from Elgin oat seeds harvested at different times during development were analyzed by SDS-PAGE under reducing conditions. The protein patterns obtained from the last four harvests closely resembled that of the mature globulin (Fig. 4). However, the first two harvests differed significantly, not only in minor components, but also in the α- and β-subunit groups themselves. During the first two weeks following anthesis, the two predominant bands of the α-subunit group had molecular weights of approximately 33,000 and 35,000. In subsequent harvests, the higher molecular weight proteins of this group (38,000 and 40,000) became prominent, and the previously described typical α-subunit group arrangement resulted. The β-subunit proteins displayed a similar phenomenon. The early predominant bands of the α-subunit group and at least one band of the β group seem to gradually disappear as the seed matures. This occurrence probably does not imply their breakdown or reduced synthesis. It likely resulted from the dilution effect associated with applying protein extracted from larger (hence, fewer) seeds of higher protein content for the last harvests. In other words, an equal representation of these bands in a mature seed globulin profile would also involve an overwhelming amount of the heavier components of the two subunit groups.

To verify whether the lower molecular weight components of the earlier harvests belonged to the globulin family, the globulin extracts were subjected to SDS-PAGE in the absence of ME (Fig. 5). The globulin patterns from all harvests showed the prominence of the higher molecular weight bands between 52,000 and 70,000 (results for harvests 1, 5, and 6 not shown). However, in the first stages of development the lower molecular weight members of the globulin family were present to a relatively greater extent. These would presumably consist of the lower molecular weight α- and β-subunit proteins described above, joined by disulfide linkages. As the seed matured, biosynthesis of the relatively higher molecular weight members prevailed. This would also explain the concomitant predominance of the heavier proteins of the two subunit groups in the later harvests (Fig. 4). Differences in the sequence of appearance of legume globulin components have also been observed (Carasco et al 1978, Khan et al 1980, Meinke et al 1981, Millerd 1975). High molecular weight bands (>100,000) were also present on SDS gels under nonreducing conditions. Because these bands also displayed lower molecular weights in the earlier stages of development (Fig. 5), they may correspond to dimers of the 52,000 to 70,000 molecular weight globulins.

Further research is required to determine whether the differential accumulation of globulins is widespread in cultivated oats and whether its timing and order of appearance varies in cultivars of different protein concentration. For example, the proportion of globulins may be maintained in a high-protein cultivar as a result of relatively precocious biosynthesis of the predominant globulin components.

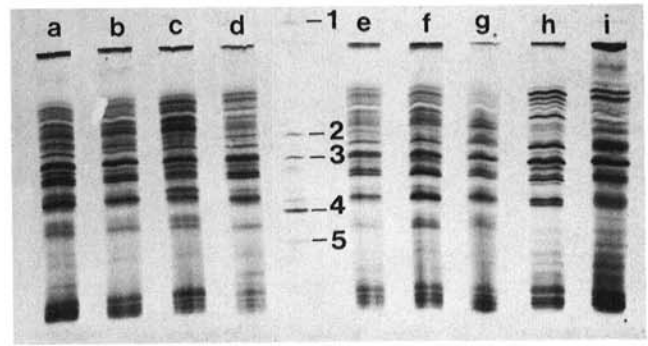

Fig. 3. Isoelectric focusing (pH 3–10) of reduced and alkylated oat globulin fractions in cultivars: a, Hinoat; b, Dal; c, Sentinel; d, Elgin; e, Harmon; F, OA-424-1; g, OT-213; h, Donald; and i, OA-269. pI Determinations done with a surface pH electrode and using pI standards: 1 amyloglucosidase pI 3.5; 2, bovine carbonic anhydrase pI 5.85; 3, human carbonic anhydrase pI 6.55; 4, horse myoglobin pI 7.35; and 5, lentil lectin pI 8.15.

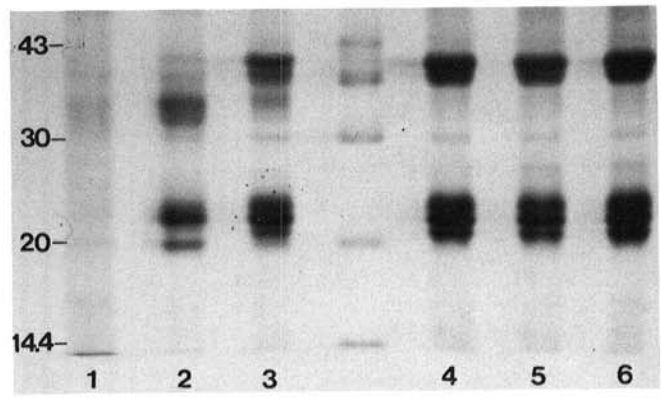

Fig. 4. Sodium dodecyl sulfate electrophoresis (with mercaptoethanol) of globulins from Elgin oat seeds harvested at weekly intervals during development. Numbers 1–6 correspond to weeks following anthesis. Molecular weight standards = ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000), and lactalbumin (14,400).

[4] K. Adeli, L. S. Robert, A. A. Urquhart, V. D. Burrows, and I. Altosaar. 1983. Nitrogen accumulation in developing oat groats from high and low protein cultivars. Unpublished data.

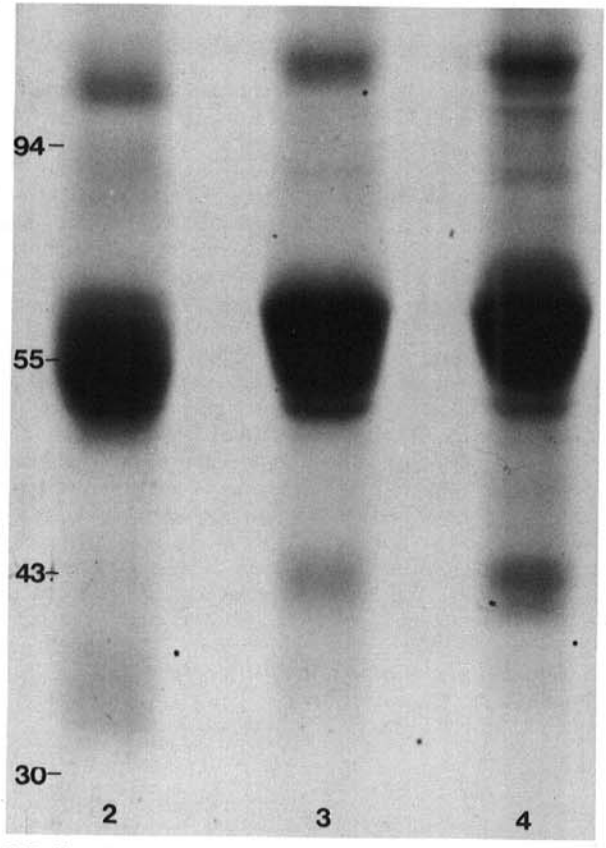

Fig. 5. Sodium dodecyl sulfate electrophoresis (without mercaptoethanol) of oat globulins at weeks 2, 3, and 4 following anthesis. Molecular weight standards = phosphorylase b (94,000), bovine serum albumin (55,000 [Matta et al 1981]), ovalbumin (43,000), and carbonic anhydrase (30,000).

CONCLUSIONS

The oat globulin fraction was shown to be quite heterogeneous, and considerable variations were observed in the electrophoretograms obtained from the nine cultivars. In addition, preliminary studies of globulin accumulation during seed development uncovered a differential order of appearance of its major constituents. In view of the relatively large phylogenetic distance separating oat and the legumes, the similarity displayed by their globulins is interesting. More research is needed to investigate the physiological implications of this conservatism and to determine whether this occurs with other cereal globulins.

After completion of this work, a similar study came to our attention (Brinegar and Peterson 1982).

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