

# Homology among 3S and 7S Globulins from Cereals and Pea

Received for publication October 19, 1984 and in revised form April 2, 1985

LAURIAN S. ROBERT, KHOSROW ADELI, AND ILLIMAR ALTOSAAR\*  
*Department of Biochemistry, University of Ottawa, Ottawa, K1N 9B4 Canada*

## ABSTRACT

The globulins from wheat caryopses were found to consist primarily of protein sedimenting at approximately 3S and 7S. These proteins displayed a molecular weight distribution similar to that of the purified vicilin-like fractions from oat and pea, with variations occurring in the isoelectric points and relative quantities of their major subunits. concanavalin A Sepharose chromatography suggested that the major polypeptides of the wheat (3S + 7S) fraction are glycosylated. Western blot analysis using antioat (3S + 7S) globulin immunoglobulin G revealed the vicilins from pea and the globulin fractions of oat, wheat, barley, rye, corn, and rice to contain immunologically homologous polypeptides. Major groups of polypeptides were shared by all the cereals and pea, including subunits of approximately 75, 50, 40 kilodaltons and 20 to 25 kilodaltons. These results indicate that legume-like 3S and 7S globulins have been conserved and are being expressed in cereals.

Major seed storage proteins of cereals and legumes generally exhibit a significant level of polypeptide microheterogeneity (6, 13). Part of this heterogeneity is believed to result from the duplication and mutational divergence of ancestral genes (10, 21). Such divergence is apparently permitted because of the weaker function-related constraints imposed on the structure of storage proteins relative to that of other seed proteins such as enzymes (16).

There are, however, limits to the evolutionary divergence of storage proteins which involve specific requirements for their recognition, processing, assembly, packaging, and degradation (6, 21). To reconcile both the existing laxity and stringency of the genetic controls involved, the observed heterogeneity might be accounted for by mutational changes permitted only in particular portions of the molecules. Examples of strong conservatism among seed storage proteins from legumes and cereals have been reported recently. These involved the two major classes of legume globulins, the legumin-like (11S) and vicilin-like (3S and 7S) fractions, and specific seed proteins from oat and rice. The major component of the oat globulin fraction was shown to be a 12S globulin bearing a very close resemblance to legumin (3, 4, 11, 17, 26). The NH<sub>2</sub>-terminal amino acid sequences of the small basic subunits from oat 12S globulin and the 11S globulins from *Glycine max*, *Vicia faba*, and *Pisum sativum* were all reported to share considerable homology (26). Similar sequences obtained from the smaller subunits of pea legumin and a purified 22-kD rice glutelin polypeptide were also demonstrated to be homologous (27). The occurrence of legumin-like proteins in cereals may indicate that, similar to legumes, vicilin-like proteins are also present. Indeed, proteins displaying similar solubility characteristics, sedimentation values, mol wt, and glycosylation patterns to that of legume vicilins have already been observed in oat (1, 4).

In this paper, we report studies on the (3S + 7S) globulin

fraction of wheat, and its homology to pea vicilins and to other cereal globulins.

## MATERIALS AND METHODS

**Plant Material.** Mature seeds of oat (*Avena sativa* L. cv Hinoat), wheat (*Triticum aestivum*, cv Fredrick), rye (*Secale cereale* cv Puma), barley (*Hordeum vulgare* cv Perth), rice (*Oryza sativa* L. cv M-101), and corn (*Zea mays* cv F64-11-6-1) were generously supplied by Dr. V. Burrows, Ottawa Research Station, Agriculture Canada. Dry pea seeds (*Pisum sativum* cv Little Marvel) were kindly provided by Dr. K. Joy, Carleton University.

**Extraction of the Total Globulin Fraction.** Peas and dehulled cereal seeds were separately milled to a flour in an electric coffee grinder. Seed globulins were extracted at room temperature with 1 M NaCl, 0.05 M Tris-HCl (pH 8.5; 3 g/100 ml, 3 h). The slurry was centrifuged (15,000g, 30 min, 24°C), and the supernatant dialyzed at 4°C against several changes of distilled water until the globulins precipitated. The globulins were recovered by centrifugation (22,000g, 45 min, 4°C) and lyophilized. In some instances, phenyl methyl sulfonyl fluoride was included in the extraction buffer; the results were not affected.

**Sucrose Density Gradient Fractionation.** The crude oat, wheat, and pea globulin fractions were dissolved in 1.0 M NaCl, 50 mM Tris-HCl (pH 8.0), and fractionated on 5 to 20% sucrose gradients made in the same buffer (4). The gradients were monitored at A<sub>280</sub> and collected into 1.5-ml fractions. The appropriate fractions were pooled and dialyzed overnight against distilled H<sub>2</sub>O. The precipitated protein was recovered by centrifugation and lyophilized.

**Gel Electrophoresis.** Two-dimensional analysis involved separation by isoelectric focusing in a pH 3.5 to 10 Ampholine (LKB) gradient followed by mol wt separation by SDS-PAGE in a 14% gel. Electrophoretic procedures were carried out as described previously (18). The mol wt markers (Pharmacia) used were: phosphorylase b (94 kD), BSA (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (20.1 kD), and  $\alpha$ -lactalbumin (14.4 kD).

**Concanavalin A Sepharose Chromatography.** Wheat total globulins were incubated with concanavalin A Sepharose (Sigma) for 30 min and the suspension poured into a column. The nonbound material was removed with 0.5 M NaCl, 0.05 M Tris-HCl (pH 7.4), and the bound proteins were eluted with 0.2 M  $\alpha$ -methyl-D-glucoside as reported previously (1). Proteins were precipitated from the bound and nonbound fractions with TCA and analyzed by SDS-PAGE. Oat 12S globulin does not bind to the column, indicating that binding is not dependent on mol wt (1).

**Antibody Preparation.** The oat (3S + 7S) holoproteins were purified from the total globulin fraction by isoelectric point precipitation and three cycles of sucrose gradient centrifugation (4). Antibodies were raised in New Zealand White rabbits, and IgG<sup>1</sup> was isolated by affinity chromatography on protein A Sepharose (11). The antibody did not cross-react with oat 12S

<sup>1</sup> Abbreviation: IgG, immunoglobulin G.

globulins (1), and the preimmune serum did not cross-react with the various globulin fractions upon immunodiffusion analysis (results not shown).

**Western Blot Analysis.** Labeling of IgG with  $^{125}\text{I}$  was performed according to a modification of the procedure utilized by Greenwood *et al.* (8). One mCi of  $^{125}\text{I}$  (low pH, high concentration; New England Nuclear) was added to 50  $\mu\text{g}$  of anti-oat (3S + 7S) globulin IgG in PBS (0.14 M NaCl, 2.7 mM KCl, 8.1 mM  $\text{Na}_2\text{HPO}_4$ , 1.5 mM  $\text{KH}_2\text{PO}_4$ , pH 7.4). Ten  $\mu\text{l}$  of 18 mM chloramine T in PBS were added and the solution incubated on ice for 10 min. Following the addition of 10  $\mu\text{l}$  of 52 mM sodium metabisulfite in PBS, the solution was applied to a 10-ml Sephadex G-25 column. The first peak fractions (containing the labeled IgG) were pooled, dialyzed against PBS, and diluted with 30 ml of 5.0% BSA, 3.0% normal rabbit serum in PBS. The specific activity was approximately 3  $\mu\text{Ci}/\mu\text{g}$  of IgG.

Western blotting was carried out similarly to the method of Towbin *et al.* (25). Immediately following SDS-PAGE, proteins were transferred electrophoretically onto a nitrocellulose sheet (0.45  $\mu\text{m}$ ; Schleicher & Schuell, BA 85) using a Hoefer transfer unit. The transfer buffer consisted of 25 mM Tris and 192 mM glycine in 20% methanol. Transfer was performed for 24 h at 135 mamp. The gel was stained and destained to verify completeness of transfer and the nitrocellulose sheet was incubated for 1 h at 40°C in 50 ml of 3.0% BSA and 3.0% NRS in PBS. After washing in PBS (2  $\times$  15 min), the sheet was incubated for 6 h in the hybridization mixture containing the  $^{125}\text{I}$ -labeled antioat (3S + 7S) globulin IgG. The nitrocellulose sheet was then washed in PBS (6  $\times$  10 min), air-dried, and exposed overnight at -70°C to Kodak X-Omat AT film in the presence of an intensifying screen.

## RESULTS

**Sucrose Density Gradient Fractionation.** The total wheat globulins were fractionated by sucrose density gradient centrifugation and the resulting pattern was compared to that of the purified oat 3S and 7S globulins (Fig. 1). Pea vicilin (7S) and legumin (11S) were used as reference standards. The sedimentation coefficients of the two major peaks observed for wheat globulins very closely match the peaks seen for the purified oat 3S and 7S globulins. These peaks represent the major fractions of the wheat

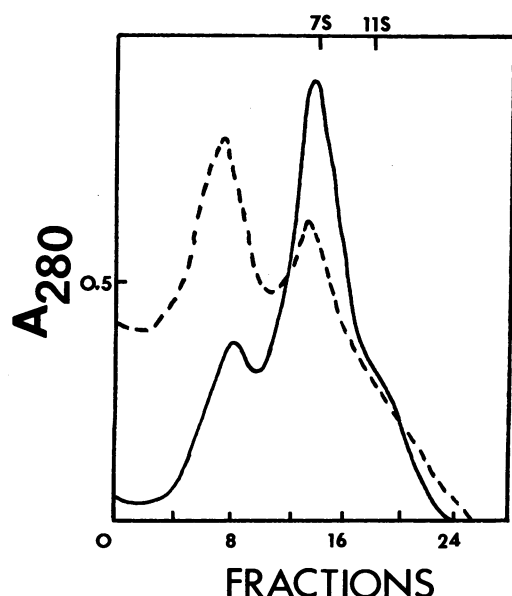


FIG. 1. Sucrose density gradient fractionation of wheat globulins and purified oat 3S and 7S globulins. (---), wheat; (—), oat. Pea vicilin (7S) and legumin (11S) holoproteins were used as reference standards.

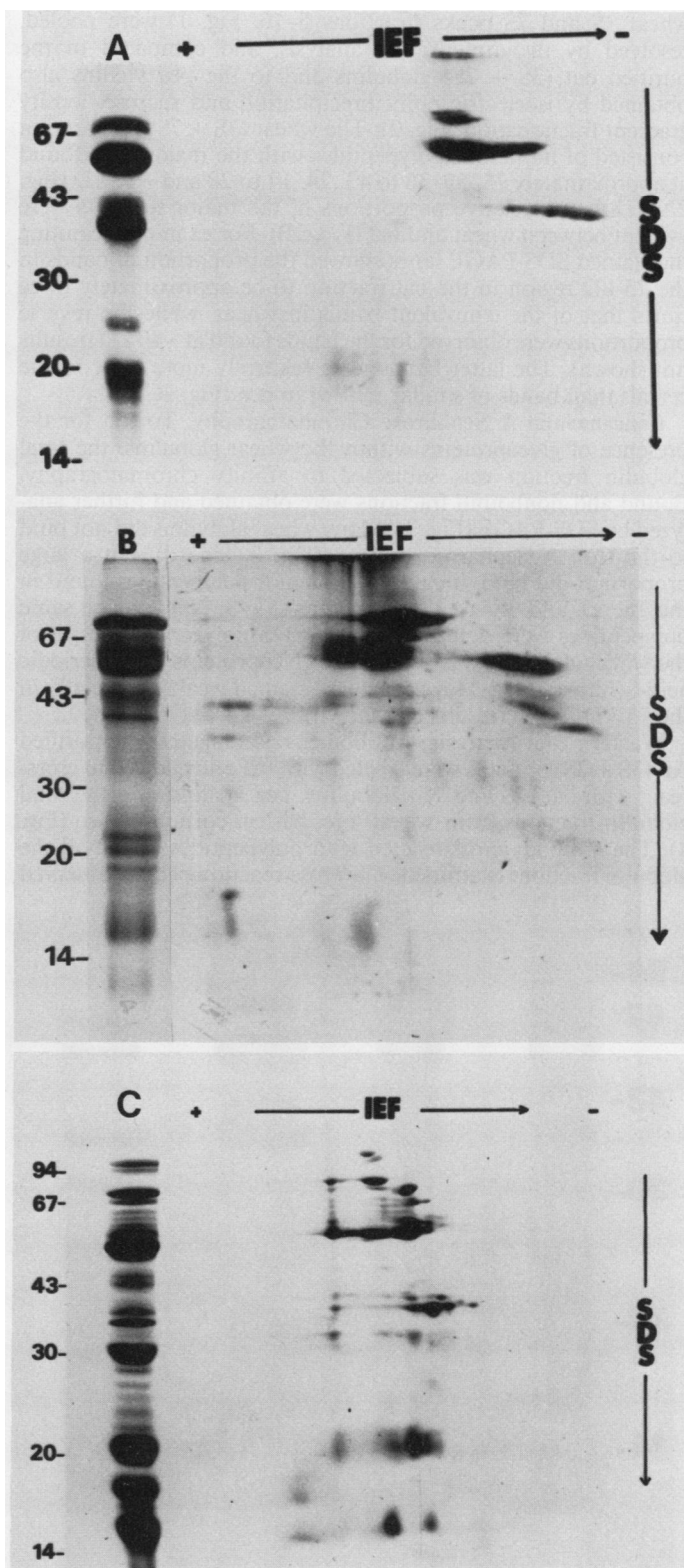


FIG. 2. Two-dimensional analysis (IEF, pH 3.6-10 followed by SDS-PAGE) of sucrose density gradient globulin fractions. A, Wheat (3S + 7S); B, oat (3S + 7S); and C, pea vicilins.

globulins whereas in oats, the 12S holoproteins constitute approximately 90% of the total globulin fraction (1). Only a small shoulder was apparent at 10-11S with wheat globulins (Fig. 1).

**Two-Dimensional Electrophoresis.** The proteins from the

wheat 3S and 7S peaks (fractions 5–16, Fig. 1) were pooled, resolved by two-dimensional analysis, and compared to the purified oat (3S + 7S) globulins and to the pea vicilins also obtained by isoelectric point precipitation and sucrose density gradient fractionation (Fig. 2). The wheat (3S + 7S) preparation consisted of numerous polypeptides with the major ones found at approximately 75, 55, 36 to 43, 24, 17 to 20 and ~10 kD (Fig. 2A). Different relative proportions of the major subunits were evident between wheat and oat (Fig. 2B). For example, scanning the stained SDS-PAGE lanes showed the proportion of bands in the 75-kD region in the oat fraction to be approximately three times that of the equivalent bands in wheat, while the reverse proportions were observed for the bands found at ~40 kD (results not shown). The latter bands were relatively more basic in the cereals than bands of similar mol wt in pea (Fig. 2C).

**Concanavalin A Sepharose Chromatography.** To test for the presence of glycoproteins within the wheat globulins, the total globulin fraction was subjected to affinity chromatography. Bound and nonbound fractions were obtained and further analyzed by SDS-PAGE (Fig. 3). Many wheat globulins did not bind to the Con A Sepharose column (Fig. 3, lane d), but a large proportion did bind, including the major polypeptides found in the 75, 50, and 36- to 43-kD regions (Fig. 3, lane c). The same polypeptides were detected when a SDS polyacrylamide gel of the wheat globulins was stained for glycoproteins using periodic acid-Schiff staining. However, these stained weakly especially in the 75-kD region (results not shown).

**Western Blot Analysis.** Antibodies raised against the purified oat (3S + 7S) proteins were labeled with  $^{125}\text{I}$  and allowed to cross-react with oat (3S and 7S) globulins, pea vicilins, and the total globulin fractions from wheat, rye, barley, corn, and rice (Fig. 4). The antibody cross-reacted with polypeptides within all the globulin fractions examined. The cross-reaction pattern observed

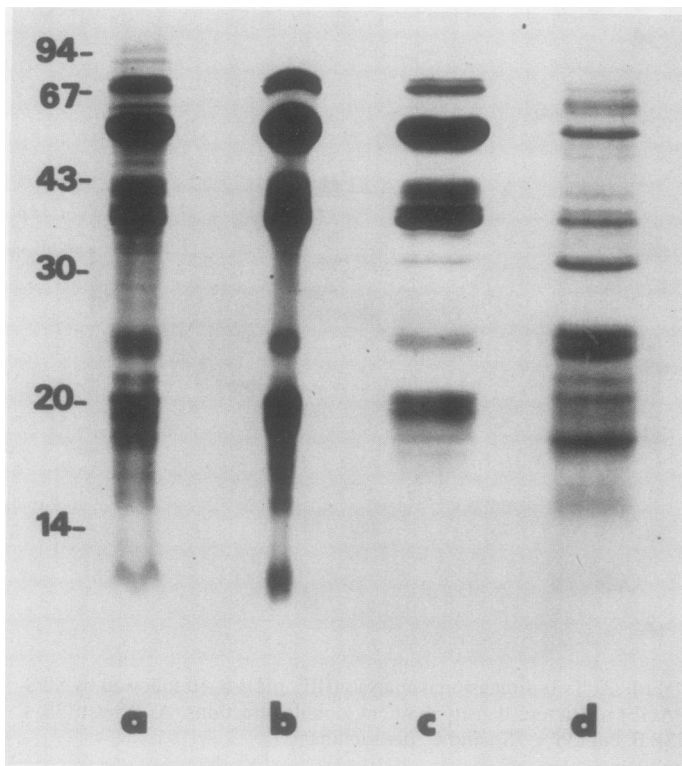


FIG. 3. SDS-PAGE of concanavalin A Sepharose chromatography fractions of wheat globulins. a, Total wheat globulins; b, wheat (3S + 7S) fraction; (c), wheat globulins which bound to the column; and (d), wheat globulins which did not bind to the column.

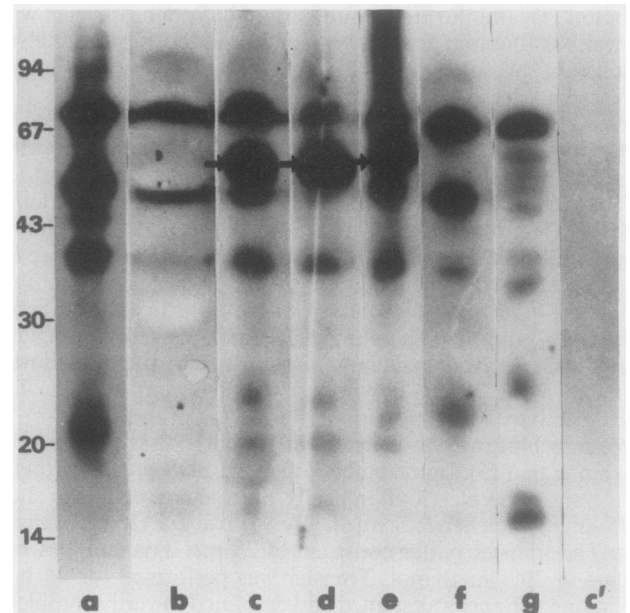


FIG. 4. Western blot analysis using  $^{125}\text{I}$ -labeled antioat (3S + 7S) globulin IgG. a, oat (3S + 7S) fraction; b, pea vicilins; c, wheat globulins; d, rye globulins; e, barley globulins; f, corn globulins; and g, rice globulins; c', control lane with no proteins.

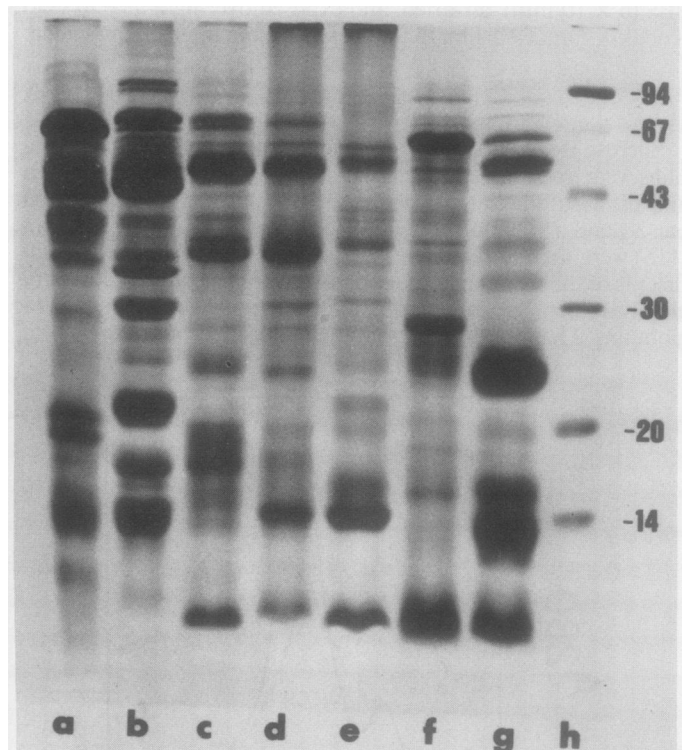


FIG. 5. SDS-PAGE of cereal globulins and pea vicilins. a, Oat (3S + 7S) fraction; b, pea; c, wheat; d, rye; e, barley; f, corn; g, rice; and h, mol wt standards.

for the wheat total globulin extract (lane c) was identical to the pattern obtained with the purified wheat (3S + 7S) globulin fraction (results not shown). When compared to the equivalent Coomassie brilliant blue-stained lanes (Fig. 5), it becomes apparent that only certain polypeptides were recognized by the antibody. However, the mol wt of the cross-reacting polypeptides within the various globulin fractions were very similar, especially

in the 40-, 50-, and 75-kD regions. Variations were observed in cross-reacting polypeptides found between 50 and 75 kD and those of mol wt less than 30 kD. Closely related species such as wheat, rye, and barley typically displayed similar cross-reaction patterns with a major band of approximately 55 kD being recognized (see arrows, Fig. 4, lanes c, d, and e).

### DISCUSSION

When fractionated by ultracentrifugation, wheat globulins consisted primarily of two major peaks with sedimentation coefficient values of approximately 3S and 7S. These results are in agreement with the sedimentation constants previously reported for wheat  $\alpha$ (2.5S) and  $\gamma$ (8.2S) globulins, respectively (5). A minor shoulder was also evident at 10-11S which probably corresponds to the wheat  $\delta$ -globulins observed by Pence and Elder (15). The polypeptides present in this 10-11S shoulder may be related to minor wheat globulins found to be antigenically homologous to 11-12S legumin-like globulins (19). The predominance of the 2-3S( $\alpha$ ) and 7-8S( $\gamma$ ) globulin fractions relative to the 10-11S( $\delta$ ) globulins in wheat is reversed in oat, where the 11S globulins largely predominate (1, 4). Barley, rye, corn, and rice, like wheat, all possess mainly  $\alpha$  and  $\gamma$  globulins, the  $\delta$ -globulins being absent or found as a minor component (5, 14). The occurrence of these broad variations in the relative levels of the (3S + 7S) and 10-12S cereal globulin fractions might implicate differential expression of the genes involved. Such variability in the content of legumin and vicilin has been observed among different genotypes of the *Pisum sativum* L. species alone (22).

Wheat (3S + 7S) globulins consisted of a heterogeneous array of polypeptides found principally at 75, 55, 36 to 43, 24, 17 to 20 and ~10 kD. Like pea, both oat and wheat displayed the considerable level of charge microheterogeneity typical of storage globulins (17, 21). They contain, however, a larger number of relatively more basic polypeptides than was observed within the pea vicilins. Gatehouse *et al.* (7) also reported that pea vicilins were generally situated between an isoelectric point range of 4.5 to 7.0. Although the mol wt distribution of pea vicilins and the (3S + 7S) globulin fractions from oat and wheat shared common features, variations in the relative quantities of the major subunits were evident. This variability among genera can be expected since different relative proportions of vicilin subunits have been observed within the single genus *Phaseolus* (6) and the single species *Pisum sativum* L. (24).

Vicilin preparations in legumes have often been shown to contain carbohydrates (6). Glycoproteins are also believed to occur in the oat (3S + 7S) globulin fraction (1). Similarly, certain polypeptides in the wheat (3S + 7S) fraction could be selected by affinity chromatography. The bound polypeptides represented all the major subunit groups. The molecular weight distribution of the affinity-selected wheat (3S + 7S) globulins was very similar to that observed with oat (1). These results also significantly resembled those obtained for pea vicilins (2), the differences arising from the absence of glycoproteins at 36 to 43 kD and their presence at ~14 kD in pea.

When challenged with radioactively iodinated anti-(3S + 7S) oat globulin IgG, polypeptides displaying common antigenic determinants were detected within the pea vicilins and the globulin fractions from wheat, rye, barley, corn, and rice. The occurrence of antigenically related polypeptides within the pea vicilin fraction is a strong indication that the (3S + 7S) globulin fraction from oat is indeed composed of proteins homologous to the equivalent fractions occurring in legumes. Therefore, the conservatism displayed among legumes and cereals involving the 10-12S legumin-like globulins (3, 4, 11, 17, 26, 27) seems to apply also to the 2-3S and 7-8S globulin. In view of such strong conservatism among these taxonomically distant dicots and monocots, one might expect to find proteins homologous to the

2-3S and 7-8S globulins within other cereals and indeed this study showed that antigenically related proteins do occur in wheat, rye, barley, corn, and rice. This of course is in agreement with the presence of  $\alpha$ (2-3S) and  $\gamma$ (7-8S) globulins in all these cereals (5, 14). It is interesting to note that unlike oat, the vicilin-like proteins predominate within the globulin fraction of cereals.

The polypeptides from the various cereals and pea which cross-reacted with the antibody displayed very similar mol wt. Major regions of homology included polypeptides found at approximately 75, 50, 40, and 20 to 25 kD. However, some differences were evident, as exemplified by the major 55-kD band occurring only in the members of the Triticeae, wheat, barley, and rye (Fig. 4, arrows). These differences can be expected among the various genera, in view of the variant subunit forms which have been observed intraspecifically, for example, with pea (12, 23) and oat (17) globulins. Such variability might also be expected to contribute to heterogeneity at the holoprotein level as shown to exist in pea (24) and soybean (9, 20). Further work is required to isolate and characterize the specific cereal globulins which cross-reacted immunologically to fully assess the extent of their homology to legume 2-3S and 7-8S globulins.

In summary, the major protein fractions of wheat globulin were shown to share similar sedimentation values, mol wt, glycosylation patterns, and antigenic determinants with the equivalent fractions of oat and pea globulins. Other cereals including barley, rye, rice, and corn were also found to contain globulins homologous to the (3S + 7S) fraction of oat. Therefore, in spite of the fact that, unlike legumes, most cereals contain prolamins and glutelins as their major seed storage proteins, globulins homologous to those occurring in legumes have been conserved and are being expressed.

### LITERATURE CITED

1. ADELI K, I ALTOSAAR 1984 Characterization of oat vicilin-like polypeptides. *Plant Physiol* 75: 225-227
2. BADENOCH-JONES J, D SPENCER, T J V HIGGINS, A MILLERD 1981 The role of glycosylation in storage-protein synthesis in developing pea seeds. *Planta* 153: 201-209
3. BRINEGAR AC, DM PETERSON 1982 Separation and characterization of oat globulin polypeptides. *Arch Biochem Biophys* 219: 71-79
4. BURGESS SR, PR SHEWRY, GJ MATLASHEWSKI, I ALTOSAAR, BJ MIFLIN 1983 Characteristics of oat (*Avena sativa* L.) seed globulins. *J Exp Bot* 34: 1320-1332
5. DANIELSSON CE 1949 Seed globulins of the Gramineae and leguminosae. *Biochem J* 44: 387-400
6. DERBYSHIRE E, DJ WRIGHT, D BOULTER 1976 Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* 15: 3-24
7. GATEHOUSE JA, RRD CROY, H MORTON, M TYLER, D BOULTER 1981 Characterisation and subunit structures of the vicilin storage proteins of pea (*Pisum sativum* L.). *Eur J Biochem* 118: 627-633
8. GREENWOOD FC, WM HUNTER, JS GLOVER 1963 The preparation of <sup>131</sup>I-labelled human growth hormone of high specific radioactivity. *Biochem J* 89: 114-123
9. HILL JE, RW BREIDENBACH 1974 Proteins of soybean seeds. I. Isolation and characterization of the major components. *Plant Physiol* 53: 742-746
10. KASARDA DD, JE BERNARDIN, CC NIMMO 1976 Wheat proteins. In Y Pomeranz, ed, *Advances in Cereal science and technology*, Vol 1. American Association of Cereal Chemists, St. Paul, pp 158-236
11. MATLASHEWSKI GJ, K ADELI, I ALTOSAAR, PR SHEWRY, BJ MIFLIN 1982 In vitro synthesis of oat globulin. *FEBS Lett* 145: 208-212
12. MATTA NK, JA GATEHOUSE 1982 Inheritance and mapping of storage protein genes in *Pisum sativum* L. *Heredity* 48: 383-392
13. MIFLIN BJ, PR SHEWRY 1979 The biology and biochemistry of cereal seed prolamins. In *Seed protein improvement in cereals and grain legumes*, Vol 1. International Atomic Energy Agency, Vienna, pp 137-156
14. MORITA Y, C YOSHIDA 1968 Studies on  $\gamma$  globulin of rice embryo. *Agr Biol Chem* 32: 664-670
15. PENCE JW, AH ELDER 1953 The albumin and globulin proteins of wheat. *Cereal Chem* 30: 275-287
16. ROBBELEN G 1979 The challenge of breeding for improved protein crops. In *Seed protein improvement in cereals and grain legumes*, Vol 1. International Atomic Energy Agency, Vienna, pp 27-42
17. ROBERT LS, GJ MATLASHEWSKI, K ADELI, C NOZZOLILLO, I ALTOSAAR 1983 Electrophoretic and developmental characterization of oat (*Avena sativa* L.) globulins in cultivars of different protein content. *Cereal Chem* 60: 231-234

18. ROBERT LS, C NOZZOLILLO, I ALTOSAAR 1983 Molecular weight and charge heterogeneity of prolamins (avenins) from nine oat (*Avena sativa* L.) cultivars of different protein content and from developing seeds. *Cereal Chem* 60: 438-442
19. ROBERT LS, C NOZZOLILLO, I ALTOSAAR 1985 Homology between legumin-like polypeptides from cereals and pea. *Biochem J* 226: 847-852
20. THANH VH, K OKUBO, K SHIBASAKI 1975 Isolation and characterization of the multiple 7S globulins of soybean proteins. *Plant Physiol* 56: 19-22
21. THOMSON JA, H DOLL 1979 Genetics and evolution of seed storage proteins. *In* Seed protein improvement in cereals and grain legumes, Vol 1. International Atomic Energy Agency, Vienna, pp 109-124
22. THOMSON JA, A MILLERD, HE SCHROEDER 1979 Genotype-dependent patterns of accumulation seed storage proteins in *Pisum*. *In* Seed protein improvement in cereals and grain legumes, Vol 1. International Atomic Energy Agency, Vienna, pp 231-240
23. THOMSON JA, HE SCHROEDER, WF DUDMAN 1978 Cotyledonary storage proteins in *Pisum sativum*. I. Molecular heterogeneity. *Aust J Plant Physiol* 5: 263-279
24. THOMSON JA, HE SCHROEDER, AM TASSIE 1980 Cotyledonary storage proteins in *Pisum sativum*. V. Further studies on molecular heterogeneity in the vicilin series of holoproteins. *Aust J Plant Physiol* 7: 271-282
25. TOWBIN H, T STAEHELIN, J GORDON 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350-4354
26. WALBURG G, BA LARKINS 1983 Oat seed globulin: subunit characterization and demonstration of its synthesis as a precursor. *Plant Physiol* 72: 161-165
27. ZHAO W-M, JA GATEHOUSE, D BOULTER 1983 The purification and partial amino acid sequence of a polypeptide from the glutelin fraction of rice grains; homology to pea legumin. *FEBS Lett* 162: 96-102