Antigenic homologies between oat and wheat globulins

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Total globulins of oat and wheat were compared by SDS-electophoresis immunoblotting and in vitro protein synthesis experiments. Numerous homologies were found. The same 4 protein subgroups, with respect to molecular mass, were found in the two cereals. Immunoblotting with antibodies for total oat globulins allowed the detection of homologous proteins in each wheat protein group, especially in the 60-kDa group, which corresponds to the major 12 S oat globulin. The same antibodies also reacted with in vitro synthesized proteins of the two cereals. However, compared to mature proteins, only a limited number of polypeptides react, suggesting that wheat globulins, like oat globulins, undergo post-translational processes.

Oat *Wheat* *Seed globulin* *In vitro translation* *Immunoblotting* *Antigenic homology*

1. INTRODUCTION

There has been considerable recent evidence that homologies exist among plant storage proteins and their related gene families [1]. We are especially interested in the cereal globulins, which are expressed to the highest degree in oat [2,3]. The major globulin from oat, a monocot, has been demonstrated to be homologous to evolutionarily distant dicot storage proteins: legumins, globulin proteins in pea, as well as soybean glycinin [4–6]. Indeed, immunological cross-reactivity is seen between rye, oat and pea globulins [7]. The amino acid profiles are similar for these globulins as well, and pea legumin is synthesized in a manner analogous to major oat globulin [6]. Similarities in the biosynthetic pathway and subunit structure of these globulins have also been reported [8].

This paper demonstrates homologies between the wheat and oat globulins. Both react with anti-oat globulin antibodies on double diffusion plates (unpublished). The antibodies were raised against native oat globulin and therefore the data suggest that there is an evolutionary conservation in the native structure of these globulins. The major oat globulin is synthesized as a 60-kDa precursor which is processed into two subunits of 40 and 20 kDa [2–8]. It is not known how wheat globulins are synthesized or if they are processed. Using oat and wheat globulins, we have studied the antigenic homologies that exist between these two cereals using proteins synthesized in vitro as well as in mature grain protein.

2. MATERIALS AND METHODS

2.1 *Plant material*

Oat (*Avena sativa* L. cultivar Hinoat) was grown at Agriculture Canada's Central Experimental Farm. Mature seed was harvested 6–8 weeks after anthesis and stored at −20°C. Wheat (*Triticum aestivum* L. cultivar Fredrick) was treated in the same manner. For oat polysome preparations, developing grains were harvested 2–3 weeks post anthesis and endosperm was collected as previously described [9]. Wheat polysomes were isolated as described elsewhere [10] from developing endosperm harvested 25 days post anthesis (cultivar Cappelle-Desprez).

2.2. *Protein extraction*

The total salt-soluble globulin fraction was

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isolated from mature seed by a method essentially similar to that previously described [11].

2.3. *Antibody production*

Rabbit immunizations against total oat globulins and purification of the anti-globulin antibodies were performed as in [2]. Antibody production was assayed by double diffusion plates [12]. The antibodies cross-reacted with both oat and wheat globulins, but not to the other Osborne fractions of either cereal.

2.4. *In vitro translations*

In vitro translations were performed using the wheat germ translation kit available from BRL. [^{35}S]Methionine, 1000 Ci/mM) was purchased from NEN. Reactions were carried out at 30°C for 1 h. Incorporation was determined trichloroacetic acid precipitation [2]. Approx. 9.75 A_{260} units of polysomes were added per 30 μl reaction mixture.

2.5. *Immunoprecipitation and gel electrophoresis*

Immunoprecipitation of in vitro synthesized products was performed by using the methods of Weber et al. [13]. SDS gel electrophoresis in 18% acrylamide gel and fluorography were performed according to [2].

2.6. *Immunoblotting*

The method Towbin et al. [14] was used, except the transfer after SDS electrophoresis in 10% acrylamide gels which was effected by transferring for 18 h in Tris-glycine buffer (pH 9.0). Anti-rabbit antibodies conjugated to horseradish peroxidase were used to detect the immunocomplexes. The peroxidase activity was revealed according to [15]. 0.05% Tween 20 was added to the incubation media to prevent non-specific reactions.

3. RESULTS

3.1. *Electrophoretic analysis*

Unreduced globulins of oat and wheat were electrophoresed on SDS slab gels. Fig.1, lane Oa, displays the SDS gel profile of unreduced oat globulins stained with Coomassie blue. The pattern can be divided into 4 main areas near 14, 20, 30–40 and 60 kDa. Some other polypeptides over 90 kDa are also revealed. The 60-kDa polypeptides represent the major protein fraction. They are the

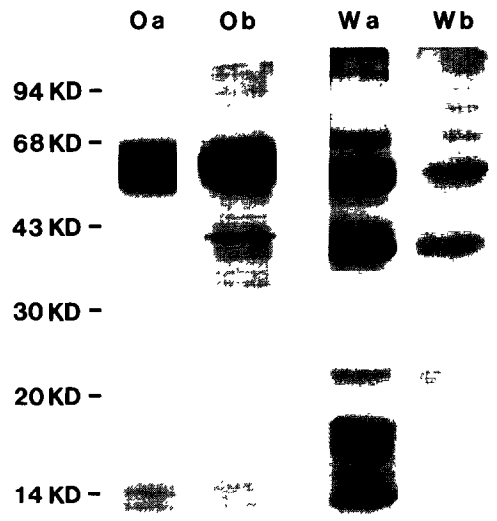

Fig. 1. SDS-polyacrylamide gel electrophoresis and immunoblotting of unreduced oat (O) and wheat (W) total globulin fractions. Oa, Wa: direct staining of the SDS gel with Coomassie blue. Ob, Wb: corresponding blots on nitrocellulose. 20 μg of oat globulins and 80 μg of wheat globulins were analysed in each corresponding track. Approximate molecular masses are indicated.

legumin-like oat globulins formed by the disulfide-linked subunits [11]. As no reducing agent was added to the extract the other areas were more faint. Nevertheless 20- and 40-kDa subunits of the 60-kDa globulins can be observed beside other salt-soluble proteins which presumbaly are oat vicilins [16,17]. In the wheat lane (fig.1, lane Wa), roughly the same 4 areas as for oat can be recognized. Compared to an oat lane obtained with an identical loading (not shown), less 60-kDa polypeptides and more 14, 20 and 40 kDa ones are observed, with additional polypeptides in the 17 and 68 kDa regions. It is of interest to note here that the major wheat globulins do not show subunit behavior when electrophoresed under reduced conditions (not shown) such as that seen when oat globulins are reduced [11].

3.2. *Immunoblotting analysis*

The original procedure of Towbin et al. [14] was inadequate to efficiently transfer all the globulins. The SDS electrophoretic separations were trans-

ferred at pH 9.0 for 18 h instead of pH 8.3 for 1 h. Under these conditions, good replicas were obtained as judged by staining nitrocellulose with amido black. These modifications allowed the analysis of the proteins of the globulin fraction. The blots were challenged with either non-immune serum or anti-oat globulin antibodies. A number of specifically stained bands using anti-oat globulin antibodies can be seen in fig.1 in both the oat (Ob) and wheat (Wb) lanes. In oat, the antibodies allowed detection of a number of polypeptides, which were similar to that obtained by Coomassie blue staining. In wheat, however, only certain polypeptides of the wheat globulin regions of the gel were immuno-stained. Specifically, the regions at 60, 40, and 14 kDa were recognized by the anti-oat globulin antibodies while most of the minor components were not stained to any large extent. It should be mentioned that 4 times more wheat protein is required for the same reactivity seen in the oat lane.

3.3. *Immunoprecipitation analysis of in vitro synthesized proteins*

Oat and wheat polysomes isolated from developing endosperm were translated in vitro in the presence of [^{35}S]methionine in a wheat germ cell-free extract. The translation mixtures were challenged with anti-oat globulin antibodies, and the resultant immunoprecipitates were analyzed by SDS-PAGE. This is shown in fig.2. Anti-oat globulin antibodies were able to immunoprecipitate proteins synthesized in vitro from both oat and wheat polysomes. However, the patterns differ significantly from that obtained by immunoblotting. In each case there is a main component surrounded by a series of less abundant polypeptides. In oat, a main component is at about 45 kDa and the minor components range from 30 to 70 kDa. In wheat, the main component is a triplet of polypeptides of about 40 kDa, surrounded by some polypeptides ranging from 30 to 50 kDa, that is molecules which are in the same molecular mass range. Additional 90-kDa components were also synthesized but no wheat component migrates in the 60-kDa region.

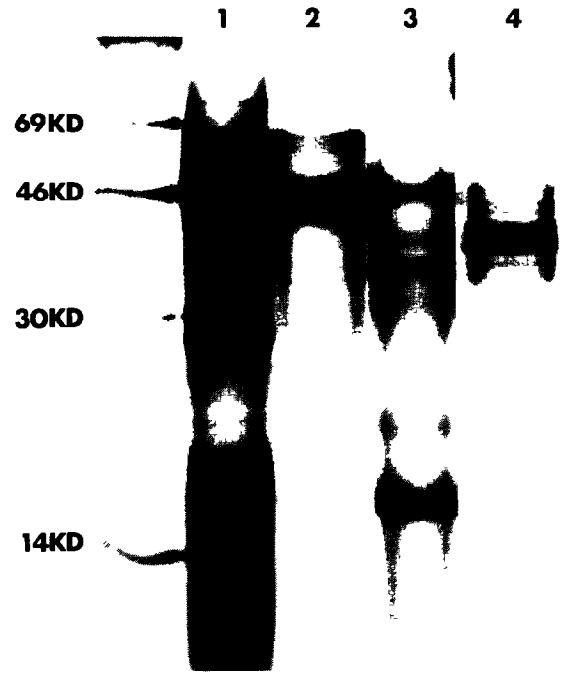

Fig. 2. In vitro synthesis of oat and wheat endosperm proteins. [^{35}S]Methionine-labelled translation products were challenged with anti-oat globulin antibodies and the precipitated proteins separated by SDS-polyacrylamide gels. Aliquots of the total translation products were also electrophoresed. Lane 1, total oat polysomes in vitro synthesis products; lane 2, immunoprecipitated oat polysome products; lane 3, total wheat polysomes in vitro synthesized products; lane 4, immunoprecipitated wheat polysome products. Approximate molecular masses are indicated.

4. DISCUSSION

The immunological homologies between wheat and oat globulins were demonstrated using both mature grain proteins and in vitro synthesized labelled proteins from both cereals. The mature proteins show similar regions of homology with respect to molecular mass on immunoblotting analysis. We have found some differences in intensity of the immune reactions. These may be due either to only partial homologies between the oat and wheat globulins or to differences in the amount of proteins present in the two species. However, the cross-reactions observed here demonstrate clearly that within the salt-soluble protein pools, homologous polypeptides are found in the two cereals. The 60-kDa region of oat globulin which consists of the disulfide-linked subunits of 40 and 20 kDa) has a homologous counterpart in wheat at 60 kDa. We can also detect

two more homologous regions in wheat at 40 and 14 kDa. It is not known whether these two lower molecular mass regions in wheat are similar to the subunit molecules in oat, or whether they represent smaller globulin species which are still homologous to oat globulins.

Polysomes of wheat and oat in in vitro experiments, directed the synthesis of a limited number of polypeptides immunoprecipitable by anti-globulin antibodies. These polypeptides are in the same molecular mass range. However, differences could be noted. For example, the 60-kDa components, which are the 12 S oat globulin precursors [2,8,18], cannot be detected in the wheat polysome translation products. This could be explained by different levels of globulin-synthesizing polysomes in the corresponding developing grains at the time of harvesting.

The patterns of the mature and in vitro synthesized globulins are somewhat different. Differences in the relative methionine content of the proteins may account for this, but one can observe that newly synthesized proteins under 30 kDa are not recognized in the two species. Since all legume globulins (either legumin or vicilin types) are known to undergo post-translational cleavages of the polypeptide chains [19], it is very plausible that wheat globulins are also submitted to delayed alterations comparable to those observed for the 60 kDa oat globulin [5]. This would explain that the small proteins are either absent or not recognized by the antibodies.

The homology between oat and wheat globulins strengthens the community of structure of these proteins [20], whatever the plant species may be, either monocot or dicot. This results from a considerable evolutionary constraint on the tridimensional structure of these molecules. It has been observed that the structure of these storage proteins is actually conserved during evolution and suggested that, in absence of any known enzymatic function, the evolutionary pressure would result from a special folding of the polypeptide chains favouring a maximal packing within the protein bodies where storage proteins are sequestered [20,21].

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