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Characterisation of high molecular weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes

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Summary. Gliadins, here defined as those proteins of defatted wheat endosperm which dissolve in 70% (v/v) ethanol at room temperature, were fractionated by gel filtration using Sephadex G-100. The protein which eluted with the void volume of the column, often described as high-molecular-weight (HMW) gliadin, was fractionated by the two different, two dimensional gel electrophoresis procedures of O'Farrell (1975) and O'Farrell et al. (1977). The next two fractions to elute from the gel column, ω-gliadin and α-, β-, γ-gliadin, were analysed similarly. The subunits of HMW gliadin and the classical (i.e. non-aggregated) gliadins map at distinctive positions on the electrophoregrams, the majority of the HMW gliadin subunits being more basic and having a slightly slower electrophoretic mobility than the α-, β-, γ-gliadins. These experiments demonstrate that those gliadins which aggregate to form HMW gliadin are distinct molecular entities and thus coded by different genes to those gliadins which do not aggregate. Glutenin, here prepared by a modification of the pH 6.4 precipitation procedure of Orth and Bushuk (1973), was also analysed by two-dimensional electrophoresis. The low-molecular-weight subunits were found to correspond exactly with the HMW gliadin subunits. Using the nullisomic-tetrasomic lines and the ditelocentric lines of 'Chinese Spring', the genes controlling the synthesis of all the major HMW gliadin subunits were shown to be located on the short arms of chromosomes 1A, 1B and 1D, as are the genes coding for the ω-gliadins and the majority of the γ-gliadins.

Key words: Gliadin – Glutenin – *Triticum* – Wheat – Endosperm

1 Introduction

Glutenin, the storage protein of wheat endosperm which imparts elasticity to a dough, has a high aggregate molecular weight, being built up of subunits which are probably linked primarily by disulphide bonds (Wall 1979). The subunits were shown by Payne and Corfield (1979) to fall into 3 groups, A, B and C, after sodium dodecyl sulphate, polyacrylamide gel electrophoresis (SDS-PAGE). The A subunits, now more usually called high-molecular-weight (HMW) subunits (Payne et al. 1982a), are well resolved by SDS-PAGE and they are being actively studied both biochemically (Field et al. 1982) and genetically (Payne et al. 1980; Lawrence and Shepherd 1981). In contrast, the smaller B and C subunits, now usually referred to as low-molecular-weight (LMW) subunits, have been studied little because they do not fractionate adequately by SDS-PAGE (Payne and Corfield 1979), or by other one-dimensional systems such as isoelectric focussing (IEF) (Kanazawa and Yonezawa 1973) or electrophoresis in aluminium lactate buffer at pH 3.1 (Huebner 1970).

The majority of aggregated storage protein (glutenin) is insoluble in neutral aqueous ethanol, in contrast to gliadin, but a small proportion of it, variously described as HMW gliadin (Beckwith et al. 1966), LMW glutenin (Nielsen et al. 1968) and Glutenin III (Graveland et al. 1982) is freely soluble. This fraction was shown by Payne and Corfield (1979) to consist of subunits which migrated like LMW glutenin subunits in SDS-PAGE gels, and to lack HMW glutenin subunits.

In this communication we report on the analysis of the LMW subunits of glutenin by the 2, two-dimensional electrophoretic procedures, used previously to analyse

in detail the HMW subunits of glutenin (Holt et al. 1981). These methods gave a much improved resolution of the LMW subunits, and enabled a critical comparison of their mobilities with those of the α-, β-, γ-gliadins. The chromosomal locations of the genes which control their synthesis were determined using genetical lines of the variety 'Chinese Spring'.

2 Materials and methods

Plant material

Field-grown grain of wheat (*T. aestivum* L.) variety 'Chinese Spring' (CS) was milled in a Bühler Mill to provide wholemeal flour for protein fractionation studies.

Some of the nullisomic-tetrasomic lines of 'Chinese Spring', developed by Sears (1954) and maintained at the Plant Breeding Institute, Cambridge, were used to study the chromosome location of the genes controlling the synthesis of the endosperm proteins. In a nullisomic-tetrasomic line a pair of chromosomes are replaced by a homoeologous pair. The following lines were analysed by two-dimensional electrophoresis:

CS N1A T1D (i.e. nullisomic 1A – tetrasomic 1D)
CS N1B T1A
CS N1B T1D
CS N1D T1B
CS N6A T6B
CS N6B T6A
CS N6D T6B

To study the chromosome arm location of LMW glutenin subunits, ditelosomic lines were examined. A ditelosomic line has the chromosome arms of each of a pair of chromosomes missing. The following lines were used:

CS DT 1AL (ditelosomic; long arms of 1A present)
CS DT 1AS (ditelosomic; short arms of 1A present)
CS DT 1BL
CS DT 1BS
CS DT 1DL

Extraction and gel-filtration chromatography of gliadin proteins from 'Chinese Spring'

Gliadin proteins were prepared from defatted wholemeal flour by the method of Payne and Corfield (1979) except that the ethanol was diluted to 70% (v/v) with 0.01 M sodium phosphate buffer, pH 7.0, instead of with water.

The gliadins were fractionated in 0.1 N acetic acid on a 2.2 cm × 80 cm column of Sephadex G-100 following the procedure of Payne and Corfield (1979). Approximately 55 A_{275} units were loaded per separation, and the eluant was collected in 7.0 ml fractions. In some column separations, 100 µl from each fraction was reduced with 2-mercaptoethanol (2-ME) and fractionated by SDS-PAGE. The fractions corresponding to each major peak of the A_{275} trace were pooled and freeze-dried to give 4 protein samples; HMW gliadin, ω-gliadin, α-, β-, γ-gliadins and albumins. The protein samples from 5 such separations were bulked, redissolved in 0.1 N acetic acid and repurified through the column before analysing by one-dimensional SDS-PAGE and two-dimensional gel electrophoresis.

Extraction and pH precipitation of glutenin (Orth and Bushuk 1973)

Gliadins and some of the glutenins were extracted from 1 g defatted 'Chinese Spring' wholemeal flour, by mixing a suspension of flour and AUC (1 : 10, w/v) (0.1 M acetic acid, 3 M urea, 0.01 M hexadecyltrimethylammonium bromide) overnight at room temperature. The slurry was then centrifuged at 20,000 g for 30 min. The pellet was discarded and the supernatant was made 70% (v/v) with respect to ethanol. After several hours at room temperature, the pH was adjusted to 6.4 with 1 N NaOH, the samples were transferred to a cold room at 4 °C, and the glutenin slowly precipitated out of solution overnight. After centrifugation at 20,000 g, the supernatant (S_1), containing mainly gliadins, was dialysed against distilled water at 4 °C for 6 days. The pellet of glutenin was purified further from the method of Orth and Bushuk (1973) by resuspending in 0.1 N acetic acid in a dual-glass homogeniser, recentrifuging, and dialysing the supernatant (S_2) as above. The gliadin (S_1) and glutenin (S_2) fractions were compared by one-dimensional SDS-PAGE and by two-dimensional electrophoresis, but the residue protein was discarded.

One-dimensional SDS-PAGE

Protein fractions were compared by SDS-PAGE using 10% gels (Payne et al. 1981). The staining and destaining procedures of Caldwell and Kasarda (1978) and Blakesley and Boezi (1977) were used.

For preparation of individual fractions of column eluant, 100 µl of sample in acetic acid was mixed with 50 µl of a more concentrated buffer than that used for grains and freeze-dried samples, to give final concentrations of 2% (w/v) SDS, 5% (w/v) 2-ME, 0.001% (w/v) Pyronin Y, 10% (v/v) glycerol and 0.063 M Tris-HCl (pH 6.8).

Two-Dimensional gel electrophoresis

a) IEF followed by SDS-PAGE (IEF×SDS-PAGE). Protein fractions and grains of genetic stocks (embryos discarded) were fractionated first by IEF and then by SDS-PAGE in a second dimension (O'Farrell 1975; Brown et al. 1979) using the procedure of Holt et al. (1981). The concentrations of the ampholines were modified slightly as follows: pH range 5–7, 0.813% was replaced by pH range 4–6, 0.406% and pH range 5–7, 0.406%.

b) Non-equilibrium pH-gradient electrophoresis (NEPHGE) followed by SDS-PAGE. The more basic proteins in the gel-filtration samples and in grains of genetic stocks were fractionated using the NEPHGE procedure described by Holt et al. (1981), which was based on the method of O'Farrell et al. (1977). In the second dimension, 17% polyacrylamide gels were used.

3 Results

3.1 Gel-filtration chromatography of gliadin

The gliadin proteins separated into five fractions (a–e) upon gel-filtration chromatography (Fig. 1). The separation is similar to that published previously by our group (Payne and Corfield 1979), and by others (Beckwith et al. 1966; Kanazawa and Yonezawa 1973). Fraction (a) corresponds to HMW-gliadin, (b) to ω-

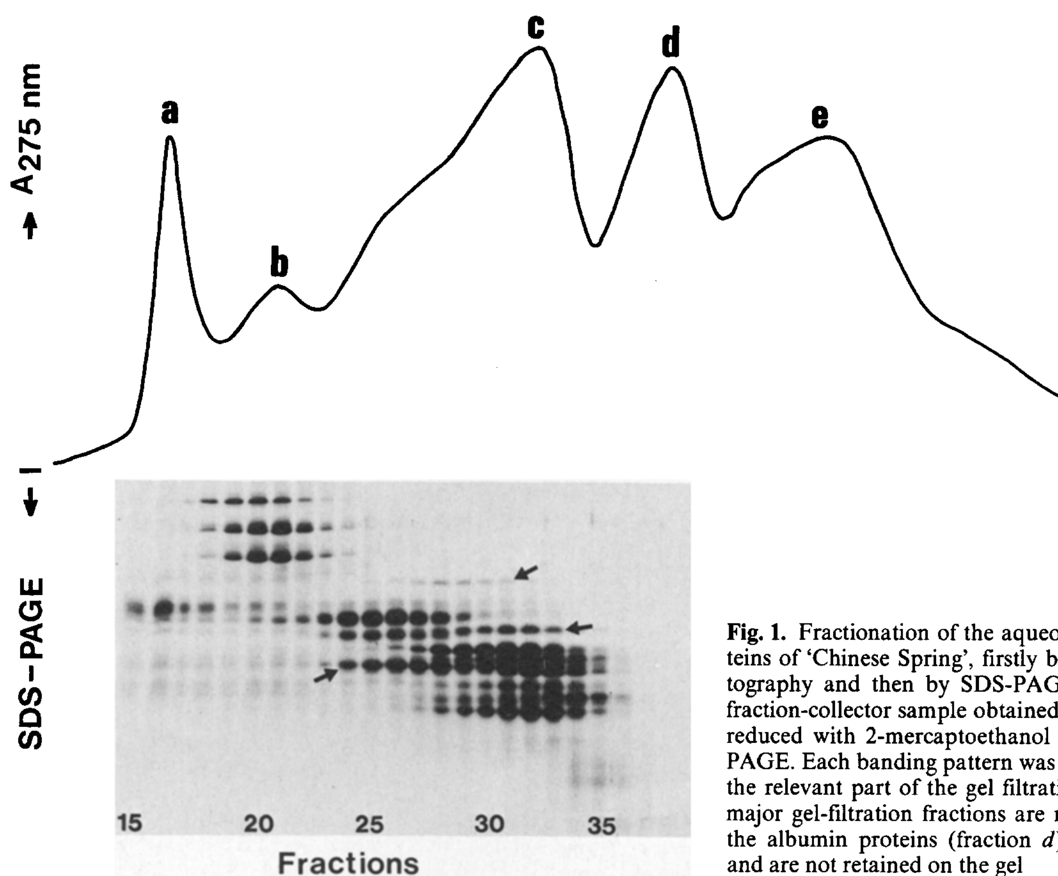

Fig. 1. Fractionation of the aqueous ethanol-soluble proteins of 'Chinese Spring', firstly by gel-filtration chromatography and then by SDS-PAGE. An aliquot of each fraction-collector sample obtained from gel filtration, was reduced with 2-mercaptoethanol and analysed by SDS-PAGE. Each banding pattern was placed directly beneath the relevant part of the gel filtration elution profile. The major gel-filtration fractions are marked (*a* – *e*). Most of the albumin proteins (fraction *d*) have a high mobility and are not retained on the gel

gliadin, (c) to a mixture of α-, β- and γ-gliadins, (d) to albumin polypeptides soluble in aqueous alcohol, and (e) to non-proteinaceous material. To analyse the change in protein composition across fractions (a) to (d), 100 μl aliquots from each of the 7 ml column fractions were reduced and subjected to one-dimensional SDS-PAGE (Fig. 1).

The major subunits of HMW gliadin of 'Chinese Spring', comprising the B-group of Payne and Corfield (1979), have a slightly slower mobility than the bulk of the α-, β-, γ-gliadins. The minor subunits (the C group of Payne and Corfield 1979) have similar mobilities to the α-, β-, γ-gliadins, but present a less complex pattern of bands. These results for the variety 'Chinese Spring' agree closely with the earlier work using the variety 'Mardler' (Payne and Corfield 1979). In general there is an inverse relationship between rate of elution from Sephadex and the mobilities in SDS-PAGE of α-, β-, γ- and ω-gliadins, although there are exceptions. The protein arrowed on the left in Fig. 1 elutes relatively early from the gel filtration column, and the proteins arrowed on the right elute much later than would be expected. The subunits of HMW gliadin appear to be present in similar proportions in all column fractions of peak (a); (Tubes 15 to 20).

The SDS-PAGE separations show that fractions (a) to (c) from the gel filtration are cross-contaminated. Accordingly, the bulked samples of 5 such separations were rechromatographed and the tube fractions which corresponded to the main peak for each experiment were kept for analysis by one-dimensional SDS-PAGE. This showed that cross-contamination had been reduced considerably (results not shown), and that the fractions were sufficiently pure to characterise in more detail by two-dimensional electrophoresis.

A composite, two-dimensional map of the storage proteins of 'Chinese Spring', constructed by placing photographs of the 2, two-dimensional systems (IEF × SDS-PAGE and NEPHGE × SDS-PAGE) side by side, is shown in Fig. 2. As the pH gradients of the IEF and the NEPHGE dimensions overlap, a small proportion of the proteins will occur on both two-dimensional separations. For clarity, the overlapping region, seen clearly in Fig. 1 and 2 of Holt et al. (1981), has been eliminated in our Fig. 2, and in later figures.

The separation, shown in Fig. 2, demonstrates the great complexity of the proteins in the wheat endosperm. About 50 major and 100 minor proteins are resolved. The two-dimensional map of the HMW gliadin subunits of 'Chinese Spring' is much simpler

Fig. 2. Combined, two-dimensional fractionation of 'Chinese Spring' endosperm proteins (NEPHGE × SDS-PAGE and IEF × SDS-PAGE). The *arrows* point to those components which are part of the HMW gliadin fraction (Fig. 3 A)

(Fig. 3 A) and the subunits fall into 3 subgroups. The major group of subunits, which correspond to the group B subunits of Payne and Corfield (1979), have a slower mobility in SDS-PAGE than the bulk of the classical gliadins (Fig. 1) and are more basic than any of the other endosperm proteins in wheat (Fig. 3 A; Fig. 2, arrows). In contrast the second group, here called D subunits, which make up only a small proportion of the total HMW gliadin subunits and were not detected by Payne and Corfield (1979), are more acidic than the other endosperm proteins (Fig. 3 A; Fig. 2, arrows). The third group which have a similar mobility to α-, β-, γ-gliadins during SDS-PAGE, and correspond to the group C subunits of Payne and Corfield (1979) have a wide range of isoelectric points, ranging from basic to neutral (Fig. 3).

The two-dimensional fractionation of the purified α-, β-, γ-gliadin preparation gave a quite different protein map (Fig. 3 B) to that of the HMW gliadin (Fig. 3 A). However, the pattern of spots due to α- and β-gliadins overlaps with the pattern of those minor C subunits of HMW gliadin which are present in the centre of Fig. 3 A, so it is difficult to identify the latter in total protein preparations. Thus, in Fig. 2, several C subunits cannot be identified and so are not arrowed, unlike the B and D subunits. The results show that HMW gliadin must have been produced by the aggregation of specific proteins in the total gliadin fraction and not by a random aggregation of a proportion of all the gliadins.

3.2 Comparison of HMW gliadin subunits with LMW subunits of glutenin

Figure 3 C illustrates a two dimensional fractionation (NEPHGE × SDS-PAGE) of glutenin prepared by a modification of the pH precipitation method of Orth and Bushuk (1973). The pattern of spots is identical to that obtained for HMW gliadin (Fig. 3 A) except for the addition of some of the HMW subunits of glutenin which are retained on the gel during NEPHGE. This indicates that the subunits of HMW gliadin and LMW glutenin are identical, as previously suspected (Payne and Corfield 1979; Bietz and Wall 1980).

3.3 Chromosomal location of the HMW gliadin subunit genes

The chromosomes which bear the genes for the HMW subunits of gliadin were investigated using the nullisomic-tetrasomic lines of 'Chinese Spring'. The ex-

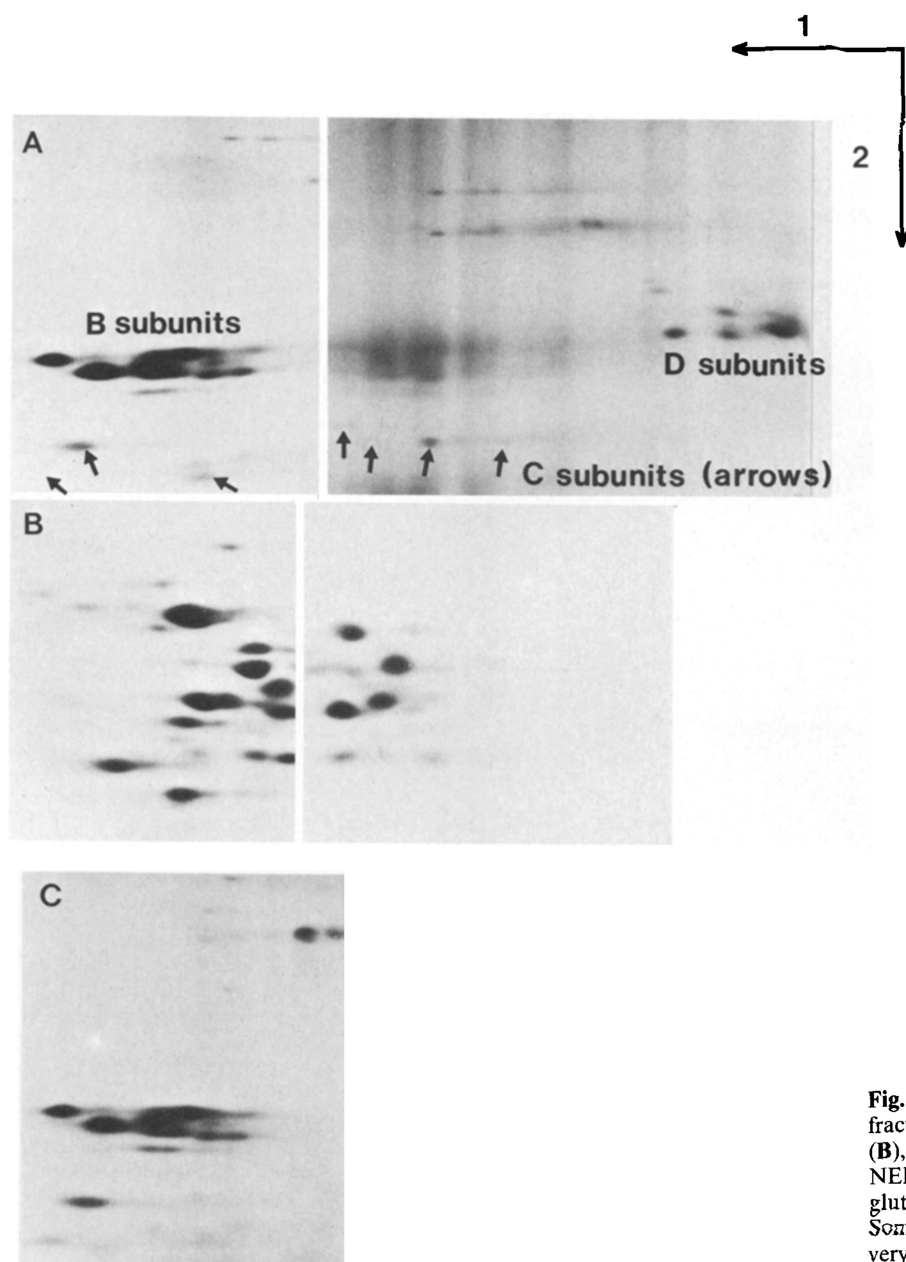

Fig. 3A – C. Combined, two-dimensional fractionation of (**A**), HMW gliadin subunits (**B**), the α-, β, γ-gliadin fraction and (**C**), NEPHGE × SDS-PAGE fractionation of glutenin prepared by pH 6.4 precipitation. *Some of the C subunits, arrowed in* **A**, are very faint

ample shown in Fig. 4 is for N1B T1A. Six spots, previously identified from Fig. 2 as HMW gliadin subunits, are missing from this line (Fig. 4, large triangles, major subunits and small triangles, minor subunits), indicating controlling genes on chromosome 1B. After the analysis of all the other group one nullisomic-tetrasomic lines, the genes for all the major HMW gliadin subunits could be assigned to either chromosome 1A, 1B or 1D. However, because other storage proteins are known to be coded by genes on the homoeologous group 6 chromosomes (Wrigley and Shepherd 1973) the corresponding nullisomic-tetrasomic series were examined for this group. The presence of the HMW gliadin subunits was not affected by any of these lines. Nullisomic-tetrasomic lines of other chromosome groups were not examined.

In Fig. 4, proteins other than HMW gliadin subunits were also lost, compared with euploid 'Chinese Spring', and these are marked as open squares for HMW subunits of glutenin, and open circles for α-, β-, γ- and ω-gliadins. Similarly in the other group 1 and also group 6 lines, other proteins were deleted. These results (not reported) are consistent with the earlier work of Brown et al. (1979) and Brown and Flavell (1981).

The arms of the group 1 chromosomes which carry the HMW gliadin subunit genes were determined by the analysis of ditelocentric lines. In DT 1BS, which

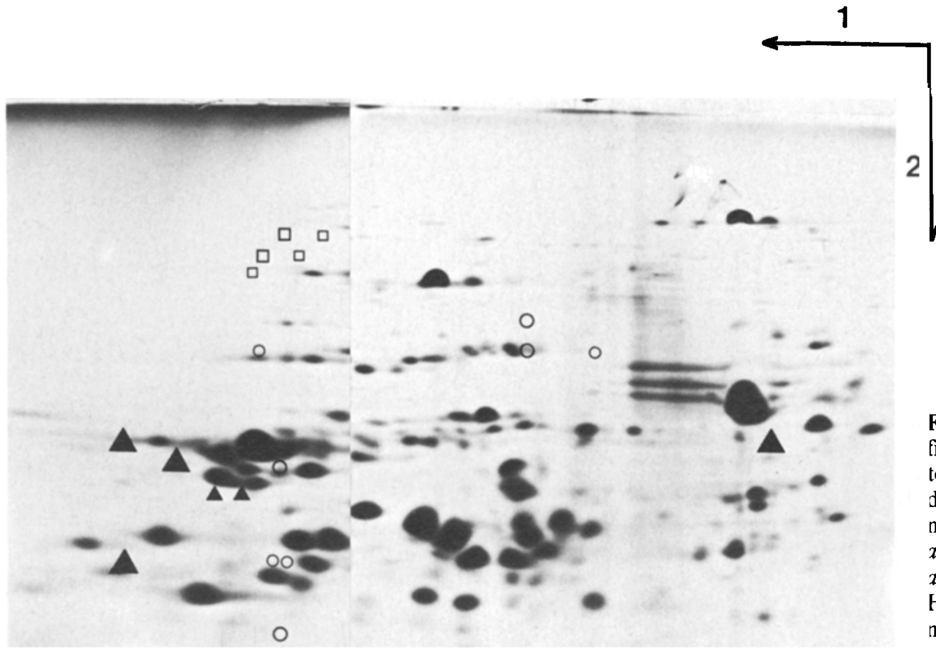

Fig. 4. Combined, two-dimensional fractionation of the endosperm proteins of CS NIB TIA. The location of deleted HMW gliadin subunits are marked by *solid triangles* (*large triangles*, major subunits and *small triangles*, minor subunits). Deleted HMW subunits of glutenin are marked by *open squares* and ω-, β-, γ-gliadins by *open circles*

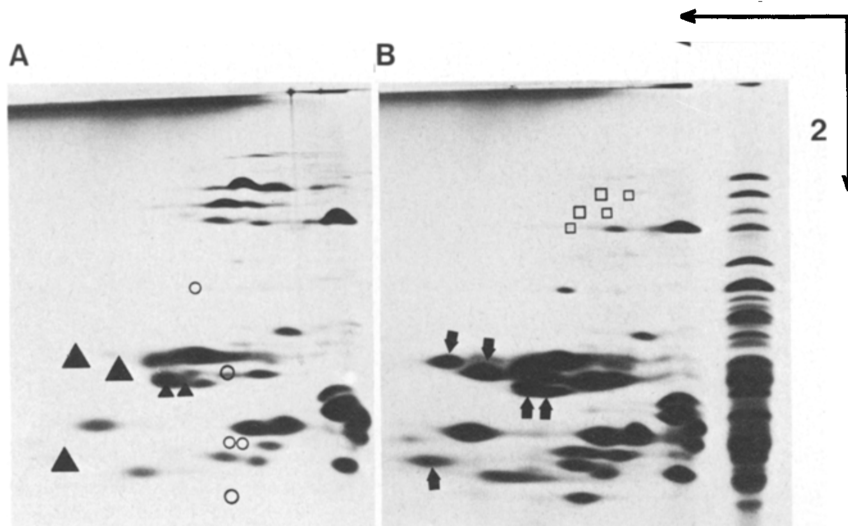

Fig. 5 A, B. Two-dimensional fractionation (NEPHGE×SDS-PAGE) of the proteins of (**A**), CS DT 1BL and (**B**) CS DT 1BS. The *arrows* in **B** point to HMW gliadin subunits known to be coded by chromosome 1B. Symbols used are the same as those in Fig. 4. On the far right a total protein extract from 'Chinese Spring' was fractionated in the second dimension only (SDS-PAGE)

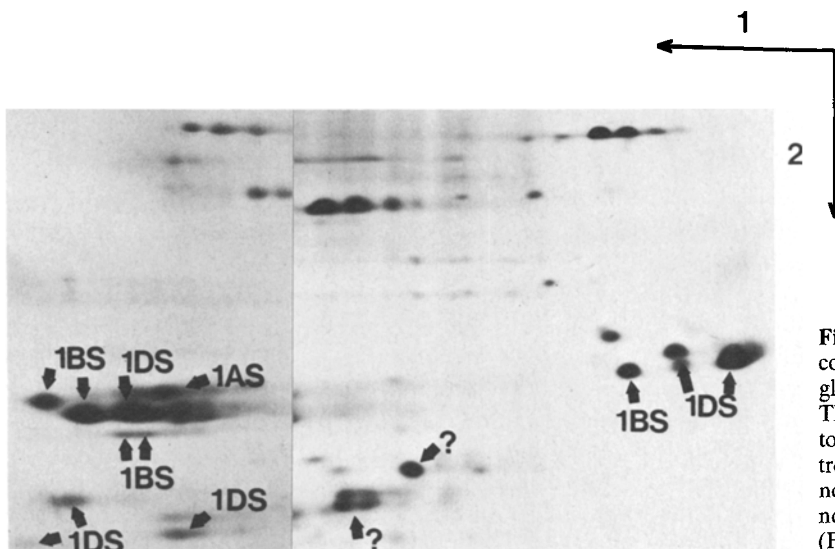

Fig. 6. Chromosome arm location of the genes controlling the synthesis of LMW subunits of glutenin/HMW gliadin subunits. *S* = short arm. The IEF part of the map has been overexposed to reveal the C subunits (Fig. 3 A). Genes controlling some of the C subunits (marked?) have not been assigned to chromosomes, as they cannot be identified in total protein preparations (Fig. 2)

lacks the chromosome 1B long arms (Fig. 5B), the HMW gliadin subunits whose genes are controlled by chromosome 1B are still expressed (Fig. 5B, arrows), but they are absent in CS DT 1BL (Fig. 5A, large and small triangles). The results demonstrate that the genes controlling all the major subunits of HMW gliadin are located on the short arms of the group one chromosomes as indicated in Fig. 6. The photographs which form Fig. 5 also show that the HMW subunits of glutenin are located on chromosome 1BL (Fig. 5B, open squares) and the ω-, γ- and β-gliadins on chromosome 1BS (Fig. 5A, open circles).

4 Discussion

Beckwith et al. (1966) showed that a small proportion (6–8%) of the ethanol-soluble gliadin proteins of wheat endosperm passes through a column of Sephadex G-100 with the void volume. This HMW gliadin fraction was thought to be in an aggregated state because of its molecular weight and its viscosity which was intermediate between that of glutenin and the classical gliadins. After a more detailed investigation by the same research group (Nielsen et al. 1968), HMW gliadin was considered to be more like glutenin and accordingly, was redefined LMW glutenin.

The structural relationship between glutenin and HMW gliadin/LMW glutenin was examined more closely by Bietz and Wall (1973). They showed that glutenin, after reduction and alkylation, became predominantly soluble in aqueous ethanol, whereas initially it was completely insoluble. The soluble subunits were shown to have a similar mobility to reduced and alkylated HMW gliadin upon SDS-PAGE. Using a more purified fraction of glutenin and a better resolving procedure of SDS-PAGE, Payne and Corfield (1979) showed that the eight electrophoretic bands of HMW gliadin in the variety 'Mardler' had exactly the same mobilities and the same relative intensities as the eight bands of LMW glutenin subunits (equivalent to the soluble subunits of Bietz and Wall 1973). Finally, Bietz and Wall (1980) showed that the proteins of these 2 groups, HMW gliadin and LMW glutenin subunits, had very similar distributions of N-terminal amino acid sequences.

From all this work it is concluded that native glutenin is a large and heterogeneous protein, built up from a collection of subunits, about 80% of them being of LMW and 20% of HMW (Payne and Corfield 1979). The larger the size of the native glutenin, the greater is the proportion of HMW subunits (Payne and Corfield 1979; Graveland et al. 1982). HMW gliadin has a similar average molecular weight to the smallest of the native glutenin molecules and it typically lacks HMW glutenin subunits, being formed entirely from subunits of LMW (Payne and Corfield 1979; Graveland et al. 1982).

In this communication the equivalence of HMW gliadin subunits and LMW subunits of glutenin is plain to see in the two-dimensional fractionations shown in Fig. 3A, C. In further discussion, these two fractions will both be called LMW glutenin subunits for convenience.

It is conceivable that some polypeptides are common to both classical gliadin and glutenin, as considered for instance by Lee (1968) and by Bietz and Rothfus (1970), since α-, β-, γ-gliadins and LMW glutenin subunits have similar electrophoretic mobilities and are both soluble in aqueous ethanol. In such a scheme, any one polypeptide in the developing endosperm could either become aggregated, via disulphide bonds, with other species of LMW and with HMW glutenin subunits to form glutenin, or form intramolecular disulphide bonds and become part of the classical gliadin fraction. Our results show that this is not the case since all the 14 major LMW glutenin subunits have different positions to the α-, β-, γ- and ω-gliadins on the composite two-dimensional map (Fig. 2), and we can conclude that they are different and distinctive proteins. This is the first time that a two-dimensional fractionation of all the LMW glutenin subunits has been published, although Brown and Flavell (1981) fractionated the minor neutral and acidic subunits by the IEF×SDS-PAGE electrophoretic procedure (C and D subunits), and obtained similar results to ours.

It is clear from the above discussions that the current definitions of gliadin and glutenin have been distorted somewhat from the original definitions of Osborne (1907) which are based entirely on solubility properties in different solvents. A fraction of Osborne's gliadin is apparently really glutenin if we define the latter as aggregated storage protein. However, if reducing agents are used for extraction, or if ethanol is acidified, or replaced by a higher alcohol such as n-propanol, or if the temperature is raised somewhat, then much of the "glutenin" becomes "gliadin". For these and other reasons, Miflin and Shewry (1979) have recommended that all the storage proteins in wheat endosperm be called gliadin. Those proteins which occur in aggregates they name as aggregative gliadin instead of glutenin (Field et al. 1982) and those which occur as free polypeptides they name as non-aggregated gliadin (that is, the α-, β-, γ- and ω-gliadins). There now seems much to support this proposal. However, evolutionary relationships between LMW glutenin subunits and gliadin polypeptides need to be understood more fully, and this will come from amino acid sequencing of purified components from each group on one side, and genetic analysis on the other.

With regard to genetics, previously only Brown et al. (1979) had separated the acidic, minor spots of LMW glutenin, and showed some to be coded by genes on the short arms of chromosome 1D. We have shown, for the first time, that the genes controlling the synthesis of the major, basic subunits of LMW glutenin are also located on the short arms of chromosomes 1A, 1B and 1D, like the genes for the ω-gliadins and the majority of the γ-gliadins (for review see Payne et al. 1982a). The only genes of LMW glutenin subunits not located to chromosomes are the minor components which migrate to similar positions on IEF×SDS-

PAGE gels as the group 6 α- and β-gliadins (Fig. 6). An analysis of allelic variation amongst LMW glutenin subunits, currently being undertaken using the group 1 and group 6 intervarietal substitution lines of 'Chinese Spring', may give further information on the location of these genes.

The ω- and γ-gliadin genes on the short arms of chromosomes 1A, 1B and 1D have already been shown to be tightly linked (Sozinov and Poperelya 1980) and to occur at the same complex loci, described as *Gli-A1*, *Gli-B1* and *Gli-D1* respectively (Payne et al. 1982b). In spite of this, the ω- and γ-gliadins are biochemically distinct: the former lack sulphur amino acids, whereas the latter are sulphur-rich (for review see Kasarda et al. 1976). The ω-gliadins also have different overall amino acid compositions, being richer in glutamine and proline (Kasarda et al. 1976), and they have markedly different N-terminal amino acid sequences (Bietz, et al. 1977; Shewry et al. 1980). Since the LMW glutenin subunits are also coded by genes on the short arms of chromosomes 1A, 1B and 1D they could either be:

i) related to the γ-gliadins
ii) related to the ω-gliadins
iii) not related either to the ω-gliadins or to the γ-gliadins.

The sulphur content, amino acid composition (Bietz and Wall 1973) and electrophoretic mobility at pH 3.1, after reduction and alkylation (Charbonnier 1973) of the major LMW glutenin subunits and ω-gliadins are different, suggesting that possibility (2) above is unlikely. In contrast, these 3 properties of LMW glutenin subunits are similar to those of the γ-gliadins, thus supporting possibility (1). However, the resemblances may only be superficial, in which case possibility (3) would be the most likely. Clearly, evolutionary relationships between low-molecular-weight subunits of glutenin and storage protein groups are not currently understood but this will almost certainly be clarified by amino-acid sequence analysis of isolated proteins. On the genetic front we have currently made crosses which will enable the genetic linkage between genes for γ-gliadin and LMW glutenin subunits to be estimated.

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References

Beckwith AC, Nielsen HC, Wall JS, Huebner FR (1966) Isolation and characterisation of a high molecular weight protein from wheat gliadin. Cereal Chem 43:14–27

Bietz JA, Huebner FR, Sanderson JE, Wall JS (1977) Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. Cereal Chem 54:1070–1083

Bietz JA, Rothfus JA (1970) Comparison of peptides from wheat gliadin and glutenin. Cereal Chem 47:381–392

Bietz JA, Wall JS (1973) Isolation and characterisation of gliadin like subunits from glutenin. Cereal Chem 50: 537–547

Bietz JA, Wall JS (1980) Identity of high molecular weight gliadin and ethanol-soluble glutenin subunits of wheat: relation to gluten structure. Cereal Chem 57:415–421

Blakesley RW, Boezi JA (1977) A new staining technique for proteins in polyacrylamide gels using Coomassie Brilliant Blue G250. Anal Biochem 82:580–582

Brown JWS, Flavell RB (1981) Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of Group 6 and Group 2 chromosomes in gliadin synthesis. Theor Appl Genet 59:349–359

Brown JWS, Kemble RJ, Law CN, Flavell RB (1979) Control of endosperm proteins in *Triticum aestivum* (var. 'Chinese Spring') and *Aegilops umbellulata* by homoeologous group 1 chromosomes. Genetics 93:189–200

Caldwell KA, Kasarda DD (1978) Assessment of genomic and species relationships in *Triticum* and *Aegilops* by PAGE and by differential staining of seed albumins and globulins. Theor Appl Genet 52:273–280

Charbonnier L (1973) Etude des protéines alcoolo-solubles de la farine de blé. Biochimie 55:1217 – 1225

Field JM, Shewry PR, March JF, Miflin BJ (1982) The purification and characterisation of homologous high molecular weight storage proteins from grain of wheat, rye and barley. Theor Appl Genet 62:329 – 336

Graveland A, Bosveld P, Lichtendonk WJ, Moonen HHE, Scheepstra A (1982) Extraction and fractionation of wheat flour proteins. J Sci Food Agric 33:1117 – 1128

Holt LM, Astin R, Payne PI (1981) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. 2. Relative isoelectric points determined by two-dimensional fractionation in polyacrylamide gels. Theor Appl Genet 60:237 – 243

Huebner FR (1970) Comparative studies on glutenins from different classes of wheat. Agric Food Chem 18:256 – 259

Kanazawa M, Yonezawa D (1973) Studies on polypeptide composition of 'low molecular weight glutenin'. Nippon Nogei Kagaku Kaishi 47:17 – 22

Kasarda DD, Bernadin JE, Nimmo CC (1976) Wheat proteins. In: Pomeranz I (ed) Advances in cereal science and technology, vol I. Plenum Press, New York, pp 158 – 236

Lawrence GJ, Shepherd KW (1981) Inheritance of glutenin protein subunits of wheat. Theor Appl Genet 60: 333 – 337

Lee JW (1968) Preparation of gliadin by urea extraction. J Sci Food Agric 19:153 – 156

Miflin BJ, Shewry PR (1979) The biology and biochemistry of cereal seed prolamins. In: Seed protein improvement in cereals and grain legumes, vol I. International Atomic Energy Agency, Vienna, pp 137–158

Nielsen HC, Beckwith AC, Wall JS (1968) Effect of disulphide-bond cleavage on wheat gliadin fractions obtained by gel filtration. Cereal Chem 45:37–47

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

O'Farrell PZ, Goodman HM, O'Farrell PH (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133–1142

Orth RA, Bushuk W (1973) Studies of glutenin. 1. Comparison of preparative methods. Cereal Chem 50: 106–114
Osborne TB (1907) The proteins of the wheat kernel. Carnegie Inst Washington Publ 84: 119 pp
Payne PI, Corfield KG (1979) Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145: 83–88
Payne PI, Corfield KG, Holt LM, Blackman JA (1981) Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J Sci Food Agric 32: 51–60
Payne PI, Holt LM, Lawrence GJ, Law CN (1982a) The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. Qual Plant Foods Hum Nutr 31:229–241
Payne PI, Holt LM, Worland AJ, Law CN (1982b) Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. 3. Telocentric mapping of the subunit genes on the long arms of the homoeolgous group 1 chromosomes. Theor Appl Genet 63: 129–138
Payne PI, Law CN, Mudd EE (1980) Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor Appl Genet 58: 113–120
Sears ER (1954) The aneuploids of common wheat. Res Bull Mo Coll Agric Exp Sta 572: 58
Shewry PR, Autran J-C, Nimmo CC, Ellen J-L, Kasarda DD (1980) N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat. Nature 286: 520–522
Sozinov AA, Poperelya FA (1980) Genetic classification of prolamines and its use for plant breeding. Ann Technol Agric 29: 229–245
Wall JS (1979) The role of wheat proteins in determining baking quality. In: Laidman DL, Wyn Jones RG (eds) Recent advances in the biochemistry of cereals. Academic Press, London, pp 275–311
Wrigley CW, Shepherd KW (1973) Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209: 154–162