Theor. Appl. Genet. 62, 329–336 (1982)

The Purification and Characterization of Homologous High Molecular Weight Storage Proteins from Grain of Wheat, Rye and Barley

J. M. Field, P. R. Shewry and B. J. Miflin
Rothamsted Experimental Station, Harpenden, Herts. (England)

J. F. March
John Innes Institute, Colney Lane, Norwich (England)

Summary. Homologous high molecular weight storage prolamins were purified from grain of wheat, rye and barley using combinations of gel filtration, ion-exchange chromatography and preparative isoelectric focusing. Sodium dodecylsulphate polyacrylamide gel electrophoresis showed that the components were single bands with apparent mol. wts. of above 100,000. Molecular weights determined by sedimentation equilibrium ultracentrifugation were considerably lower; 54,700, 67,600 and 69,600 for the components from barley, rye and wheat respectively. Amino acid analysis showed the presence of 13.6 to 16.5 mol% glycine, 29.6 to 34.0 mol% glutamate + glutamine, 11.4 to 13.7 mol% proline and a total of 4.0 to 5.7 mol% basic amino acids. Automated N-terminal amino acid sequencing of the component from wheat showed the presence of cysteine residues at positions 5 and 10, and this is discussed in relation to the possible role of these proteins in the visco-elastic gluten network.

Key words: HMW storage prolamins – Wheat – Rye – Barley – Characterization and purification

Introduction

Wheat gluten proteins can be solubilised by solutions containing acetic acid, urea and cetyltrimethyl ammonium bromide and separated by gel permeation chromatography into two major fractions (Meredith and Wren 1966; Huebner and Wall 1976; Field et al. 1982a). The fraction eluting in the void volume of such columns is a disulphide-linked aggregate with a mol. wt. equivalent to or greater than an extended molecule of 1.2×10^6 daltons (Field et al. 1982a) and has been termed "glutenin". The second major fraction elutes within the separation range of the column and consists of a series of alcohol-soluble polypeptides which are usually called gliadins. Gliadins are characterized by a high content of glutamine and proline and a low content of basic amino acids (Kasarda et al. 1976) and such alcohol-soluble proteins (or prolamins) are the major storage protein components of most cereal seeds including wheat, barley, rye, and maize (Miflin and Shewry 1979).

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) of reduced 'glutenin' shows that it is composed of a group of high molecular weight (HMW) polypeptides (apparent mol. wts. in the range 90,000 to 144,000) and other polypeptides with apparent mol. wts. in the same range as gliadin (around 36,000 to 44,000) (Payne and Corfield 1979; Field et al. 1982a). The former have been termed HMW subunits by Payne and coworkers (Payne et al. 1979, 1981a, b; Holt et al. 1981) while chemical analysis of the latter components indicates that they are closely related to gliadin (Bietz and Wall 1973, 1980).

We have suggested that the HMW components of the glutenin fraction are also prolamin storage proteins for the following reasons. 1) They can be partially extracted in aqueous alcohols (particularly 50% v/v propan-1-ol) at room temperature and are almost completely extracted if a reducing agent and 1% acetic acid are included in the solvent (Miflin et al. 1980). 2) Amino acid analysis shows that they are rich in glutamate + glutamine and proline and poor in lysine, but they differ from other prolamins in containing 12–20% glycine (Khan and Bushuk 1979). 3) They are present in protein bodies isolated from developing grain (Miflin et al. 1981), where, as in gluten, they are almost entirely present in the form of high mol. wt. aggregates (Field et al. 1982b).

Two lines of evidence indicate that the HMW subunits are important in determining breadmaking quality. Firstly, the amounts of the aggregates, in which they are major components, are directly correlated with baking quality (Field et al. 1982a) and secondly, the presence of certain subunits is positively correlated with baking quality in the progeny of crosses between some European cultivars (Payne et al. 1979, 1981a).

Genetic studies have shown that HMW subunits are coded for by loci on the long arms of the homoeologous chromosomes of group 1 (Bietz et al. 1975; Payne et al. 1980,

0040-5752/82/0062/0329/$ 1.60

1981b; Holt et al. 1981; Lawrence and Shepherd 1980, 1981a, b). Recent studies indicate that similar polypeptides are also present in related cereal species. Lawrence and Shepherd (1981a) presented genetic evidence for the presence in rye and barley of related polypeptides coded for by loci on the long arms of chromosomes 1R and 5 respectively. Further we have prepared high mol. wt. aggregates from protein bodies of developing grain of these two species and shown that they contain components with similar migration on SDS-PAGE to the HMW subunits of wheat (Field et al. 1982b).

In this paper we report the purification and analysis of HMW components of the prolamin storage proteins of wheat, barley and rye. Their characteristic amino acid compositions confirm the genetic evidence that they are homologous while further detailed analysis of the wheat polypeptide allows some suggestions to be made as to structure-function relationships within the gluten network.

Materials and Methods

Chemicals

Stock solutions of 8M urea were deionised on a column of Biorad AG 501–X8D mixed bed ion-exchange resin. Ampholytes and Ultradex for isoelectric focusing were obtained from LKB. 4-vinyl pyridine was obtained from Sigma and redistilled before use. Cyanogen bromide was obtained from Pierce and 5,5′-dithiobis-(2-nitrobenzoic acid) from Sigma.

Protein Extraction

Seed of wheat (cv. 'Highbury'), rye (cv. 'Rheidol') and barley (cv. 'Sundance') were milled in a Glen Creston Hammer Mill to pass a 0.7 mm sieve. The rye and barley meals were extracted to remove phenols and other non-prolamin alcohol-soluble compounds by stirring for 30 min with 5 ml/g of 95% ethanol. A total secalin fraction was then extracted from rye by stirring for 2×1 h at 20 °C with 5 ml/g of 50% (v/v) propan-1-ol+2% (v/v) 2-mercaptoethanol. Hordein was extracted from barley in two sequential fractions by stirring for 1 h at 20 °C with 5 ml/g of 50% (v/v) propan-1-ol (to extract hordein-I) followed by 50% (v/v) propan-1-ol+2% (v/v) 2-mercaptoethanol (to extract hordein-II). The hordein-II fraction is enriched in the HMW component and was used for the subsequent purification. Prolamins were extracted from wheat in two sequential fractions. The first was extracted by stirring for 2 h at 4 °C with 5 ml/g of 70% (v/v) ethanol+1% (v/v) 2-mercaptoethanol. This contained mainly the lower molecular weight α, β, γ and ω-gliadins and was discarded. The second fraction, which was enriched in HMW subunits, was extracted by stirring overnight at room temperature with 5 ml/g of 50% (v/v) propan-1-ol+2% (v/v) 2-mercaptoethanol+1% (v/v) acetic acid.

Prolamins were precipitated from the supernatants (after neutralisation of the wheat supernatant with NaOH) by the addition of 1 or 2 volumes of aq sodium chloride to a final concentration of 1 M. After standing at 4 °C overnight the precipitated prolamins were collected by centrifugation and lyophilized.

Total prolamin fractions for sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) were prepared by sequential extractions of 1 g meal with 2×20 ml of water saturated butan-1-ol for 30 min each at 20 °C to remove lipids, 3×20 ml of 0.5 M NaCl for 1 h each at 20 °C to remove salt-soluble proteins followed by 3×20 ml of 50% (v/v) propan-1-ol+2% (v/v) 2-mercaptoethanol+1% (v/v) acetic acid for 1 h each at 60 °C to extract prolamins (as described by Shewry et al. 1980b). The prolamin extracts were dialysed against water and then lyophilized.

Alkylation

Prolamins were reduced and pyridylethylated or carboxymethylated by the procedures of Friedman et al. (1970) and Crestfield et al. (1963) respectively.

Gel Filtration

The enriched gliadin preparation was fractionated by gel filtration on a 90×8 cm column of Sephacryl S300 in 6 M urea, 0.01 M acetic acid and containing 1% (v/v) 2-mercaptoethanol when the fraction was unalkylated.

Ion-Exchange Chromatography

1 g protein was dissolved in 100 ml of 10 mM glycine/acetate buffer, pH 4.6, containing 3 M urea and the components separated by chromatography on a column of CM cellulose with a 0–0.2 M NaCl gradient in the same buffer (Shewry et al. 1981).

Preparative Isoelectric Focusing

Preparative isoelectric focusing in a flat bed of Ultradex granulated gel was carried out on an LKB Multiphor using the method of Winter et al. (1975) modified by the incorporation of urea to solubilise the proteins. 5 g of Ultradex was mixed with 5 ml of ampholyte (pH range 5–9 for barley and rye, 3.5 to 10 for wheat) 75 ml 8 M urea, 25 ml H_2O and 10 mM dithiothreitol for separation of unalkylated proteins and poured into the gel tray. It was then dried under a current of air until the first signs of crystallization of urea on the surface of the gel. Up to 200 mg protein was dissolved in 10 ml of 6 M urea, 10 mM glycine, pH adjusted to 8.0 with Tris and dialysed against the same buffer. The sample was loaded by mixing with the gel in a zone close to the anode. Focusing was for 24 h at 10 W and 2 °C. Zones of gel were eluted in small columns with 3 M urea, 0.01 M acetic acid. The eluates were dialysed, lyophilized and freed of ampholyte by washing with absolute ethanol followed by desalting on Sephadex G75 in 0.1 M acetic acid.

Electrophoresis

Proteins were separated in gels containing 12.5% acrylamide at pH 8.9 as described by Shewry et al. (1978). Molecular weight standards were as described previously (Shewry et al. 1981).

Amino Acid Analysis

1 mg samples of protein were hydrolysed for 21 h at 110 °C under N_2 with 1 ml of 6 N HCl containing 0.1% 2-mercaptoethanol. Amino acids were determined by automated cation

exchange chromatography using a modified Technicon TSM-1 amino acid analyser (Shewry et al. 1978). Cysteine was determined as its pyridylethyl derivative. Tryptophan was not determined. Results presented are means of duplicate hydrolyses and analyses and those for serine and threonine are increased by 10% and 5% respectively to correct for destruction during hydrolysis.

Amino Acid Sequencing

N-terminal amino acid sequencing was carried out with a Beckman 890 C automatic sequencer. Carboxymethylated or pyridyethylated protein (ca. 5–10 mg) was applied to the sequencer in 50% (v/v) acetic acid. A quadrol buffer system was used with double coupling on the first degradation cycle. The resultant thiazolinones were converted to the phenylthiohydantoin amino acids (Edman and Begg 1967). The PTH amino acids were identified using gas liquid chromatography (Pisano et al. 1972), thin layer chromatography (Kulbe 1974), and high-performance liquid chromatography (HPLC).

A Water Associates gradient system was used with a C.18, 5 micron packing in an 8 mm diameter cartridge. The separated PTH amino acids were monitored at 269 nm and 313 nm. The separation was carried out in the reverse phase mode using a 0.03 M sodium acetate buffer, pH 4.7, and acetonitrile two solvent system. Solvent 'A' was composed of 90% buffer and 10% acetonitrile and solvent 'B' of 10% buffer and 90% acetonitrile. The proportion of solvent 'B' was increased from 15% to 100% during the elution.

Cysteine Cleavage

The cyanylation reagent, 2-nitro-5-thiocyanobenzoic acid, was prepared either as the half potassium salt from KCN and 5,5'-dithiobis-(2-nitrobenzoic acid) or directly as the free acid using NaCN in 0.5 M Tris acetate buffer (pH 8.2) instead of KCN in $KHCO_3$ solution (Degani and Patchornik 1971). Purity of the recrystallised product was assessed by melting point determination.

An aliquot (10 mg) of unalkylated subunit 2 from Highbury was dissolved in 10 ml of 6 M guanidinium chloride in 0.2 M Tris-acetate buffer pH 8.3, containing 0.3 mM DTT and incubated at 37 °C for two hours. A five-fold molar excess of 2-nitro-5-thiocyanobenzoic acid over total thiol was then added and the solution allowed to stand for 15 min at room temperature, after which the pH was adjusted to 9.0 with 1 M NaOH and the cleavage reaction effected by overnight incubation at 37 °C. The procedure described is essentially that of Jacobson et al. (1973) with the exception that the total thiol concentration was reduced as recommended by Degani and Patchornik (1974) to maximise the extent of cyanylation. The cleaved material was then dialysed against 3 M urea at 4 °C to remove guanidinium chloride prior to the addition of SDS to a concentration of 1% and subsequent PAGE. A sample of unalkylated Highbury prolamin (extracted with 50% n-propanol-1% acetic acid-2% 2-mercaptoethanol) was modified and cleaved in an identical manner. In both cases a portion of the cyanylated material was withdrawn before the cleavage reaction for SDS-PAGE.

Sedimentation Equilibrium Ultracentrifugation

Aliquots of prolamin preparations were dissolved in 6 M guanidinium hydrochloride – 0.1 M sodium acetate (pH 4.0) at concentrations ranging from 0.1 to 0.2 mg ml^{-1} and dialysed exhaustively at 4 °C against several changes of the same solvent. Sedimentation analyses were performed at 20 °C in an MSE Centriscan 75 ultracentrifuge, equipped with photoelectric scanner and multiplexor, by the meniscus depletion technique (Yphantis 1964) using 12 mm single sector cells and a column height of 3 mm. Data were analysed according to the procedure of Roark and Yphanthis (1969). Partial specific volumes were calculated from compositional data and corrected for preferential interactions with guanidine according to Lee and Timasheff (1979).

Results and Discussion

SDS-PAGE of Total Prolamins

SDS-PAGE (Fig. 1) showed the presence of HMW bands in the total prolamin fractions of all three species. In Highbury three major and two minor bands were present and these are numbered (in order of increasing mobility) subunits 2, 6, 17, 18 and 12 according to the nomenclature of Payne and coworkers (Payne et al. 1981 b; Holt et al. 1981) who have shown that subunits 2 and 12 are coded by chromosome 1D, and subunits 6, 17 and 18 by chromosome 1 B. Payne and Corfield (1979) estimated the apparent mol.wts. of these bands as between 90,000 and 144,000. Bands of similar mobility were also present in secalin from rye but these were less clearly resolved. The poor resolution probably resulted from the presence in each band

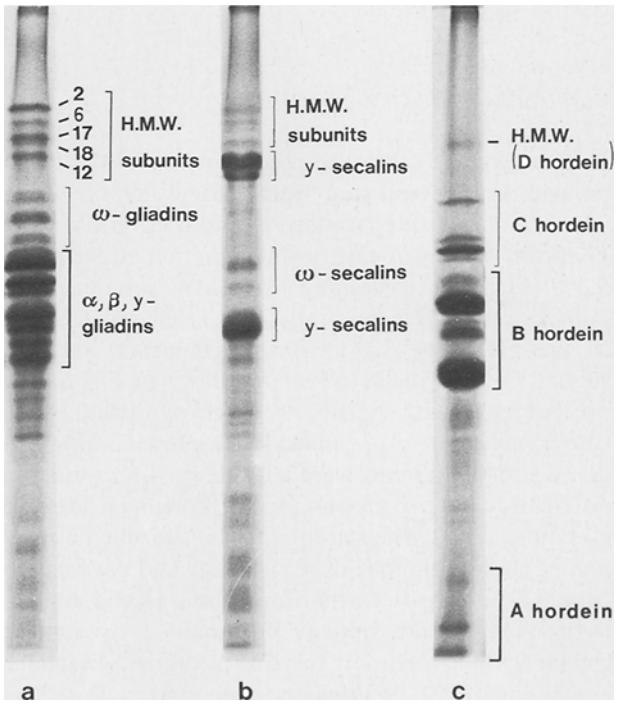

Fig. 1a–c. SDS-PAGE of reduced and pyridylethylated total prolamin fractions from wheat (**a**), rye (**b**) and barley (**c**)

of a number of components with slightly different mobilities. This is because rye is a species which usually outcrosses and as a result most cultivars are mixed populations which may vary in their storage protein components. Analysis by SDS-PAGE of secalin fractions extracted from single seeds shows the presence of heterogeneity in the patterns of HMW bands in a number of cultivars including Rheidol which was used in this study (Shewry et al. 1982 b). In contrast to rye and wheat only a single HMW band is present in the hordein fraction of barley cv. 'Sundance'. We have shown that a single band of similar mobility is present in other European cultivars, but one band of slightly faster mobility or occasionally one major and one minor band are present in other lines from the world barley collection (Shewry and Miflin 1982). The other components of the hordein fraction are classified into three groups called 'A', 'B', and 'C' hordein in order of decreasing mobility (Køie et al. 1976; Miflin and Shewry 1977) (Fig. 1). We have therefore designated the HMW components as 'D' hordein. Comparison of the hordein, secalin and gliadin fractions separated in Fig. 1 reveals considerable variation in the proportion of HMW bands. Such SDS-PAGE separations are difficult to quantify with different procedures sometimes giving widely varying results. However, two different gel scanning procedures based on dye binding (Coomassie BBR 250) and light scattering both showed that HMW bands accounted for at least twice as much of the total prolamin fraction in wheat as in barley or rye (Miflin et al. 1982).

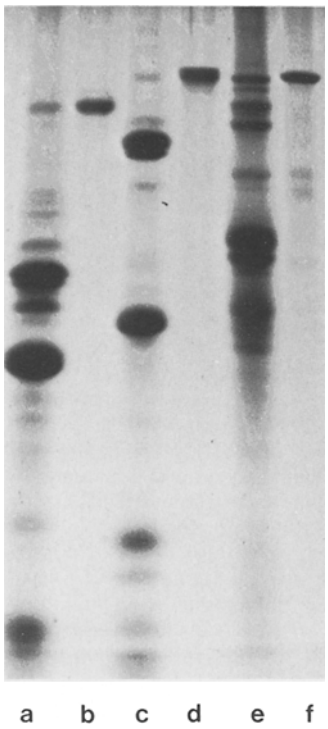

Fig. 2 a–f. SDS-PAGE of purified HMW components and the prolamin fractions used as starting material for the purifications. **a** hordein II fraction of barley; **b** HMW hordein component ('D' hordein); **c** total secalin fraction of rye; **d** HMW secalin component; **e** enriched gliadin fraction of wheat; **f** HMW subunit 2. All samples are reduced and pyridylethylated

Purification of HMW Components

Different procedures were used to purify HMW components from wheat and from barley and rye. With wheat a gliadin preparation, enriched in HMW subunits, was first fractionated by gel filtration on Sephacryl S 300. Fractions containing the HMW subunits were bulked and subjected to preparative isoelectric focusing in the pH range 3.5 to 10, which clearly resolved subunit 2 from all the others as shown in Fig. 2. The procedure worked equally well for pyridylethylated, carboxymethylated or unalkylated protein, although the pI's of the subunits were affected by the addition of differently charged groups to the protein by the two alkylating agents. The starting materials for the purification of HMW components from barley and rye were an enriched hordein-II fraction and total secalin respectively. These were initially fractionated by ion-exchange chromatography on CM cellulose. Fractions containing HMW components were bulked from several chromatographic runs and re-chromatographed using the same procedure. The partially purified components were then separated by preparative isoelectric-focusing in the pH range 5–9. This gave a highly purified preparation of the barley component (Fig. 2) and a rye preparation which gave a single diffuse band on SDS-PAGE, indicating the presence of more than one polypeptide (Fig. 2). Only a very low yield of this component was obtained.

Amino Acid Compositions

Amino acid analyses of the three components showed very similar compositions (Table 1) notably high glycine (13.6 to 16.5 mol%) and glutamate + glutamine (29.6 to 34%), relatively low proline compared to other prolamins (11.4 to 13.7%) and low amounts of basic residues (a total of 4.0 to 5.7%). There were also some differences, notably more alanine in the wheat and rye components and more threonine and serine in the barley.

The composition determined for the wheat subunit is in good agreement with those reported for two purified HMW subunits by Khan and Bushuk (1979). The large amount of glycine is clearly characteristic of this group of prolamins as this amino acid is only a minor component (less than 5%) of other prolamins from the three species (see Miflin et al. 1982).

Table 1. Amino acid compositions of HMW components from barley, rye and wheat

	Wheat	Rye	Barley
Asp[a]	2.79	1.30	1.50
Thr	3.25	2.72	8.00
Ser	6.45	5.01	9.43
Glu[1]	32.60	34.05	29.65
Pro	12.82	13.72	11.38
Gly	14.85	16.46	13.61
Ala	5.03	6.43	3.42
Cys[2]	0.84	1.42	1.68
Val	3.53	2.77	4.81
Met	0.73	0.29	0.60
Ile	1.72	1.61	1.24
Leu	5.27	4.43	3.89
Tyr	4.43	4.12	3.76
Phe	1.65	0.92	1.31
His	0.86	2.16	3.12
Lys	1.36	0.25	0.82
Arg	1.83	2.34	1.78

Results are expressed as mol %
[a] include the amides asparagine and glutamine
[b] determined as pyridylethylcysteine

Molecular Weights

Comparison of the mobilities of the three components on SDS-PAGE with those of standard proteins indicated that all had apparent mol. wts. in excess of 100,000. However, the molecular weights determined by sedimentation equilibrium ultracentrifugation were considerably lower: 69,600 for subunit 2 from wheat, 67,600 for the rye component and 54,700 for the barley component. Assuming mean residue weights of 107.5, 106.3 and 106.8 (calculated from the data in Table 1) it can be calculated that the wheat, rye and barley components contained approximately 647, 636 and 512 residues per mole respectively.

The overestimation of mol. wts. by SDS-PAGE has previously been reported for gliadin (Hamauzu et al. 1975), hordein (Shewry et al. 1980b) and secalin (Shewry et al. 1982a). However, since hordein, gliadin (Hamauzu et al. 1974; Bietz and Wall 1972; Shewry et al. 1980b) and glutenin (Bietz and Wall 1972) all bind similar amounts of SDS per gram to other proteins such as ovalbumin and serum albumin this cannot be due to decreased detergent binding. Hamauzu et al. (1975) suggested that the anomalous results were caused by the effects of the high proline content on the conformation of the protein/SDS complex and Duhamel et al. (1980) have recently reported that proline-rich animal proteins (calf thymus histone H1, collagen and procollagen) also have anomalously low mobility on SDS-PAGE.

Cysteine Cleavage of Wheat Subunit 2

Selective cleavage at cysteine can be achieved using the procedure of Jacobson et al. (1973) and this technique was used to determine whether the cysteine residues in subunit 2 were located close to one or both ends of the polypeptide chain. The unalkylated subunit was purified, cyanylated and the modified cysteine residues cleaved by incubation at pH 9. Comparison, by SDS-PAGE, of the mol.wts. of the uncleaved and cleaved preparations (Fig. 3b, c) showed that only a small increase in mobility occurred on cleavage, indicating only a slight decrease in mol.wt. This is consistent with the location of all of the cysteine residues close to one or both ends of the polypeptide. When a total gliadin preparation from Highbury was subjected to cysteine cleavage there was a similar small increase in the mobility of all the HMW bands indicating a similar terminal location of the cysteine residues (Fig. 3d, e). The migration of the ω-gliadins was not affected which is consistent with the reported absence of cysteine from these components (Charbonnier 1974; Kasarda et al. 1982) while the S-rich α, β and γ gliadins were not observed in the cleaved preparation suggesting that they were extensively fragmented.

N-terminal Amino Acid Sequences

The N-terminal amino acid sequence of wheat subunit 2 was determined by automated Edman degradation.

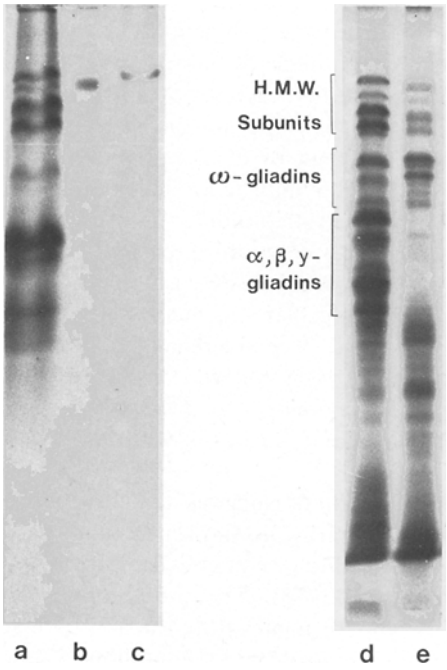

Fig. 3a–e. The effect of cleavage at cysteine residues on the migration on SDS-PAGE of total gliadin and purified HMW subunit 2. **a** cyanylated total gliadin; **b** cyanylated and cysteine-cleaved HMW subunit 2; **c** cyanylated HMW subunit 2; **d** cyanylated total gliadin; **e** cyanylated and cysteine-cleaved total gliadin

Table 2. N-Terminal amino acid sequence of HMW Subunit 2 of wheat cv. 'Highbury'

1	2	3	4	5	6	7
NH_2 – GLU –	GLY –	GLU –	ALA –	(CYS) –	GLU –	GLN –

8	9	10	11	12	13	14	15	16
– LEU –	GLN –	CYS –	GLN or GLU	– ? –	GLU or PRO	– LEU –	GLN –	PRO

The sequence obtained (Table 2) is notable for the presence in the first 16 residues of two cysteines out of a total of 5 or 6 in the whole polypeptide.

The sequence is not related to N-terminal sequences previously reported for α, β and γ gliadins of bread wheat (Bietz et al. 1977); ω-gliadin of *Triticum monococcum* and C-hordein of barley (Shewry et al. 1980a). When the 'D' hordein component of barley was subjected to the same procedure no release was obtained indicating that the N-terminus was blocked. N-terminal blocking has also been reported for 'B' hordein (Shewry et al. 1980b; Schmitt and Svendsen 1980), but not as yet for any prolamins of rye or wheat. We were unable to prepare sufficient quantities of the rye component for N-terminal sequence determination.

A Role for HMW Subunits in Gluten Structure

Two types of theory have been proposed as to the molecular basis of the visco-elastic properties of wheat gluten. In the first, the major stabilizing forces are disulphide bonds between the polypeptide chains and the linear glutenin hypothesis of Ewart (1968, 1972, 1977, 1978) is one of the first theories of this kind. He proposed that elasticity is due to the presence of glutenin polypeptides joined by disulphide bonds into long linear polymers with only a limited amount of branching, and suggested that additional secondary forces built up sequentially to produce tension, particularly during work-hardening, while viscous flow depended primarily on molecular slip but also partly on mechanical scission and S-S interchange. Theories which differ in some respects but which all stress the importance of disulphide bonds have been proposed by Bloksma (1975), Khan and Bushuk (1978, 1979) and Wall (1979).

An alternative hypothesis suggests that disulphide bonds are present primarily as intra-molecular links and that aggregation is due to non-covalent forces (see for example Bernardin 1978). The most convincing support for this hypothesis comes from the ability of A gliadin to aggregate into fibrils under conditions of low pH and low ionic strength (Kasarda et al. 1967). However, a considerable body of evidence points to the importance of disulphide bonds, notably the existence of aggregates, as revealed by gel filtration, in the presence of agents which disrupt hydrogen and hydrophobic bonds (such as 8 M urea, detergents and aqueous alcohols) and the destruction of these aggregates by reducing agents (Field et al. 1982a).

The properties demonstrated here for subunit 2 make it an ideal component of an elastic disulphide-linked aggregate. Firstly, the high glycine content confers flexibility on the polypeptide chain since the spectrum of permitted bond angles for this residue and thus the conformational space available to it is greater than for any other amino acid (see Schulz and Schirmer 1979). Secondly the presence of 5 or 6 cysteine residues confers the ability to form disulphide linked aggregates. Two of these were present in the short N-terminal sequence reported here and cysteine cleavage indicated that all were located close to one or both ends of the polypeptide chain.

Although it remains to be demonstrated that one or more of the cysteine residues are located close to the C-terminus, their presence in this region would enable the polypeptide to form long head-to-tail polymers as proposed in Ewart's linear glutenin hypothesis.

The relationship between the properties of the HMW subunits and the genetically determined differences in the baking qualities of different cultivars is as yet unclear. Although there is a close correlation between baking quality and the relative amount of glutenin recovered in high molecular weight aggregates, varieties appear to have similar total amounts of HMW subunits which are major components of these aggregates (Field et al. 1982a). Thus it is probable that the basis of varietal differences lies either in the intrinsic properties of the different HMW subunits which affect their ability to form aggregates or in the activity of enzyme systems which control disulphide bond formation.

Conclusions

The characteristic amino acid compositions reported here for the HMW components from wheat, rye and barley confirms the genetic evidence (Lawrence and Shepherd 1981a) that these are homologous proteins. We have also shown (Miflin et al. 1981, 1982; Field et al. 1982b) that HMW components are present in protein bodies prepared from developing endosperms of all three species. This indicates that they have a role as storage proteins rather than as structural components as suggested for the HMW subunits of wheat by Wall (1979). The HMW components of all three species are also soluble in aqueous alcohols although reduction to break down the aggregates may be necessary. This, together with their prolamin-like amino acid compositions, leads us to define them as aggregative prolamins rather than as glutelins.

Acknowledgements

The authors are grateful to Dr. P. I. Payne for advice and discussion on the HMW subunits of wheat and to Dr. J. R. S.

Ellis (RHM Research) for supplying seed of rye. We also wish to thank Saroj Parmar for assistance with SDS-PAGE analyses and Susan Smith for amino acid analyses. The work was supported by grants from the Home Grown Cereals Authority and the EEC (No. 470).

Literature

Bernardin, J.E. (1978): Gluenin protein interaction with small molecules and ions – the control of flour properties. Bakers Digest **52,** 20–23

Bietz, J.A.; Huebner, F.R.; Sandersen, J.E.; Wall, J.S. (1977): Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. Cereal Chem. **54,** 1070–1083

Bietz, J.A.; Shepherd, K.W.; Wall, J.S. (1975): Single kernal analysis of glutenin: use in wheat genetics and breeding. Cereal Chem. **52,** 513–532

Bietz, J.A.; Wall, J.S. (1972): Wheat gluten subunits: molecular weights determined by sodium dodecylsulphate polyacrylamide gel electrophoresis. Cereal Chem. **49,** 416–430

Bietz, J.A.; Wall, J.S. (1973): Isolation and characterization of gladin-like subunits from glutenin. Cereal Chem. **50,** 537–547

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

Bloksma, A.H. (1975): Thiol and disulphide groups in dough rheology. Cereal Chem. **52** (3, II), 170r–183r

Charbonnier, L. (1974): Isolation and characterization of ω-gliadin fractions. Biochim. Biophys. Acta **359,** 142–151

Crestfield, A.M.; Moore, S.; Stein, W.H. (1963): The preparation and enzymatic hydrolysis of reduced and S-carboxymethylated proteins. J. Biol. Chem. **238,** 622–627

Degani, C.Y.; Patchornik, A. (1971): Selective cyanylation of sulphydryl groups. II on the synthesis of 2-nitro-5-thiocyanobenzoic acid. J. Org. Chem. **36,** 2727–2728

Degani, Y.; Patchornik, A. (1974): Cyanylation of sulphydryl groups by 2-nitro-5-thiocyanobenzoic acid. High yield modification and cleavage of peptides at cysteine residues. Biochemistry **13,** 1–11

Duhamel, R.C.; Meezan, E.; Brendel, K. (1980): Metachromatic staining with Coomassie Brilliant Blue R250 of the proline-rich calf thymus histone H1. Biochim. Biophys. Acta **626,** 432–442

Edman, P.; Begg, G. (1967): A protein sequenator. Eur. J. Biochem. **1,** 80–81

Ewart, J.A.D. (1968): A hypothesis for the structure and rheology of glutenin. J. Sci. Food Agric. **19,** 617–623

Ewart, J.A.D. (1972): A modified hypothesis for the structure and rheology of glutenins. J. Sci. Food Agric. **23,** 687–699

Ewart, J.A.D. (1977): Re-examination of the linear glutenin hypothesis. J. Sci. Food Agric. **28,** 191–199

Ewart, J.A.D. (1978): Glutenin and dough tenacity. J. Sci. Food Agric. **29,** 551–556

Field, J.M.; Shewry, P.R.; Miflin, B.J. (1982a): Solubilization and characterization of wheat gluten proteins; correlations between the amount of aggregated proteins and baking quality. J. Sci. Food Agric. (submitted for publication)

Field, J.M.; Shewry, P.R.; Burgess, S.R.; Forde, J.; Parmar, S.; Miflin, B.J. (1982b): The presence of large molecular weight protein aggregates in the protein bodies of developing wheat and other cereal grains. J. Cereal Sci. (subm. for publ.)

Friedman, M.; Krull, L.H.; Cavins, J.F. (1970): The chromatographic determination of cystine and cysteine residues in proteins as S-β-(4-pyridylethyl)cysteine. J. Biol. Chem. **245,** 3868–3871

Hamauzu, Z.; Kamazuka, Y.; Kanazawa, H.; Yonezawa, D. (1975): Molecular weight determination of component polypeptides of glutenin after fractionation by gel filtration. Agric. Biol. Chem. **39,** 1527–1531

Hamauzu, Z.; Toyomasu, T.; Yonezawa, D. (1974): Studies on sodium dodecyl sulphate complex of reduced gliadin in relation to the abnormality in SDS-polyacrylamide gel electrophoresis. Agric. Biol. Chem. **39,** 1407–10

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

Huebner, F.R.; Wall, J.S. (1976): Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. **53,** 258–269

Jacobson, G.R.; Schaffer, M.H.; Stark, G.R.; Vanaman, T.C. (1973): Specific chemical cleavage in high yield at the amino peptide bonds of cysteine and cystine residues. J. Biol. Chem. **248,** 6583–6591

Kasarda, D.D.; Autran, J.C.; Lew, E.J-L.; Nimmo, C.C.; Shewry, P.R. (1982): N-terminal amino acid sequences of ω-gliadin and ω-secalins: implications for the evolution of prolamin genes. Biochim. Biophys. Acta. (subm. for publ.)

Kasarda, D.D.; Bernard, J.E.; Thomas, R.S. (1967) Reversible aggregation of α-gliadin to fibrils. Science **155,** 203–205

Kasarda, D.D.; Bernardin, J.E.; Nimmo, C.C. (1976): Wheat proteins. In: Advances in Cereal Science and Technology I. (ed. Pomeranz, Y.), pp. 158–236. St. Paul, Minnesota: Am. Assoc. Cereal Chem.

Khan, K.; Bushuk, W. (1978): Glutenin: structure and functionality in breadmaking. Bakers Digest **52,** 14–20

Khan, K.; Bushuk, W. (1979): Studies of glutenin XIII gel filtration, isoelectric focusing, and amino acid composition studies. Cereal Chem. **56,** 505–512

Køie, B.; Ingversen, J.; Andersen, A.J.; Doll, H.; Eggum, B.O. (1976): Composition and nutritional quality of barley protein. In: Evaluation of Seed Protein Alterations by Mutation Breeding, pp. 55–61. Vienna: IAEA

Kulbe, K.D. (1974): Micropolyamide thin layer chromatography of phenylthiohydantoin amino acids (PTH) at subnanomolar level. A rapid microtechnique for simultaneous microsample identification after automated Edman degradations. Anal. Biochem. **59,** 564–573

Lawrence, G.J.; Shepherd, K.W. (1980): Variation in glutenin protein subunits of wheat. Aust. J. Biol. Sci. **33,** 221–233

Lawrence, G.J.; Shepherd, K.W. (1981a): Chromosomal location of genes controlling seed proteins in species related to wheat. Theor. Appl. Genet. **59,** 25–31

Lawrence, G.J.; Shepherd, K.W. (1981b): Inheritance of glutenin protein subunits of wheat. Theor. Appl. Genet. **60,** 333–337

Lee, J.C.; Timasheff, S.N. (1979): Calculations of protein partial specific volumes. In: Methods in enzymology, vol. **61,** (eds. Hirs, C.H.W.; Timasheff, J.S.), pp. 49–57. New York: Acad. Press

Meredith, O.B.; Wren, J.J. (1966): Determination of molecular weight distribution in wheat flour proteins by extraction and gel filtration in a dissociating medium. Cereal Chem. **43,** 169–186

Miflin, B.J.; Burgess, S.R.; Shewry, P.R. (1981): The development of protein bodies in the storage tissues of seeds. Subcellular separations of homogenates of barley, maize and wheat endosperms and of pea cotyledons. J. exp. Bot. **32,** 199–219

Miflin, B.J.; Byers, M.; Field, J.M.; Faulks, A.J. (1980): The isolation and characterization of proteins extracted from whole milled grain, gluten and developing protein bodies of wheat. Ann. Technol. Agric. **29,** 133–147

Miflin, B.J.; Field, J.M.; Shewry, P.R. (1982): Cereal storage proteins and their effect on technological properties. In: Proc. Seed Protein Symp. Versailles

Miflin, B.J.; Shewry, P.R. (1977): An introduction to the extraction and characterization of barley and maize prolamins. In: Techniques for the Separation of Barley and Maize Seed Proteins (eds. Miflin, B.J.; Shewry, P.R.), pp. 13–21. Luxembourg: EEC

Miflin, B.J.; Shewry, P.R. (1979): The biology and biochemistry of cereal seed prolamins. In: Seed Protein Improvement in Cereals and Grain Legumes, Vol. 1, pp. 137–158. Vienna: IAEA

Payne, P.I.; Corfield, K.G. (1979): Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta **145,** 83–88

Payne, P.I.; Corfield, K.G.; Blackman, J.A. (1979): Identification of a high molecular weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet. **55,** 153–159

Payne, P.I.; Corfield, K.G.; Holt, L.M.; Blackman, J.A. (1981a): 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, P.I.; Holt, L.M.; Law, C.N. (1981b): Structural and genetical studies of the high-molecular-weight subunits of wheat glutenin. Part 1. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor. Appl. Genet. **60,** 229–236

Payne, P.I.; Law, C.N.; Mudd, E.E. (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

Pisano, J.J., Bronzert, T.J., Brewer, H.B., Jr. (1972): Advances in gas chromatographic analysis of amino acid phenyl- and methylthiohydantoins. Anal. Biochem. **45,** 43–59

Roark, D.E.; Yphantis, D.A. (1969): Studies of self-associating systems by equilibrium ultracentrifugation. Ann. N.Y. Acad. Sci. **164,** 245–278

Schmitt, J.M.; Svendsen, I. (1980): Amino acid sequence of hordein polypeptides. Carlsberg Res. Commun. **45,** 143–148

Schulz, G.E.; Schirmer, R.H. (1979): Principles of Protein Structure. Berlin: Springer Verlag

Shewry, P.R.; Hill, J.M.; Pratt, H.M.; Leggatt, M.M.; Miflin, B.J. (1978): An evaluation of techniques for the extraction and separation of hordein and glutelin from barley seed and a comparison of the protein composition of Bomi and Risø 1508. J. exp. Bot. **29,** 677–692

Shewry, P.R.; Autran, J.-C.; Nimmo, C.C.; Lew, E.J-L.; Kasarda, D.D. (1980a): N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat. Nature **286,** 520–522

Shewry, P.R.; Field, J.M.; Kirkman, M.A.; Faulks, A.J.; Miflin, B.J. (1980b): The extraction, solubility and characterization of two groups of barley storage polypeptides. J. exp. Bot. **31,** 393–407

Shewry, P.R.,; Field, J.M.; Lew, E.J-L., Kasarda, D.D. (1982a): The purification and characterization of two groups of storage proteins (secalins) from rye (*Secale cereale* L.). J. exp. Bot. **33,** 261–268

Shewry, P.R.; Lew, E.J-L., Kasarda, D.D. (1981): Structural homology of storage proteins coded by the *Hor 1* locus of barley (*Hordeum vulgare* L.). Planta **153,** 246–253

Shewry, P.R.; Miflin, B.J. (1982): Genes for the storage proteins of barley. Qualitas Plantarum (Plant Foods for Human Nutrition) (in press)

Shewry, P.R.; Parmar, S.; Miflin, B.J. (1982b): The extraction, separation and polymorphism of the prolamin storage proteins (secalins) of rye. Cereal Chem. (in press)

Wall, J.S. (1979): The role of wheat proteins in determining baking quality. In: Recent Advances in the Biochemistry of Cereals (eds. Laidman, D.L.; Wyn Jones, R.G.), pp. 275–311. London: Acad. Press

Winter, A.; Perlmutter, H.; Davies, H. (1975): Preparative flat-bed electrofocusing in a granulated gel with the LKB 2117 Multiphor. LKB Application Note No. 198. Sweden: LKB Produkter AB

Yphantis, D.A. (1964): Equilibrium ultracentrifugation of dilute solutions. Biochemistry **3,** 297–317

Received March 19, 1982
Communicated by R. Riley

Dr. J. M. Field
Dr. P. R. Shewry
Dr. B. J. Miflin
Rothamsted Experimental Station
Harpenden, Herts. (England)

Dr. J. F. March
John Innes Institute
Colney Lane
Norwich (England)