Variation in Glutenin Protein Subunits of Wheat

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Abstract

The high molecular weight glutenin protein subunits (those with apparent molecular weight in the range 80000 to 140000) of 98 wheat cultivars have been examined using a discontinuous gel-electrophoresis system. The number of bands present in each cultivar ranged from three to five and at least 34 different band patterns were observed. Examination of these patterns revealed that some bands, or band combinations, are mutually exclusive and that they can be assigned to three groups. In one group, two pairs of bands occur as alternatives and these bands are controlled by genes on chromosome 1D of wheat. In the second group, three possibilities occur with cultivars possessing either one of two single bands or neither band. These patterns are controlled by a gene or genes on chromosome 1A. In the third group nine patterns occur, four consisting of single bands and five consisting of a pair of bands. Four of these nine patterns have been shown to be controlled by genes on chromosome 1B. The variation detected in the glutenin subunits is useful for cultivar identification, has a bearing on our understanding of wheat evolution, and raises questions concerning the nature of this variation.

Introduction

The unique cohesive–elastic properties of doughs made from flour of hexaploid wheat (Triticum aestivum) result primarily from the properties of the gluten proteins which make up about 85% of the endosperm storage proteins (Krull and Wall 1969; Kasarda et al. 1971). Gluten is insoluble in water or dilute salt solutions but about half of it (the gliadin fraction) is soluble in solutions containing 70% ethanol (Osborne 1907) or 2 M urea (Holme and Briggs 1959). Gel electrophoresis has shown that the gliadin fraction contains at least 40 different protein species (Wrigley and Shepherd 1973) which frequently show extensive variation between cultivars (e.g. Zillman and Bushuk 1979). However, differences in gliadin composition revealed by gel electrophoresis have not been related to differences in properties of doughs or baking quality of flour (Orth and Bushuk 1972; Tanaka and Bushuk 1972; Wrigley and Shepherd 1973).

The gluten fraction insoluble in 70% ethanol (the glutenins) may be of particular importance in determining gluten properties because it contains protein aggregates of high molecular weight (up to several million) formed by the association of a number of constituent polypeptide chains (see Kasarda et al. 1976 for a review). These polypeptides are referred to as subunits in this paper following the nomenclature adopted by previous workers in this field. The glutenin aggregates can be broken down into their component subunits by treatment with an agent which breaks disulfide bonds, such as 2-mercaptoethanol (2-ME), and an agent
which disrupts, either directly or indirectly, hydrophobic interactions and hydrogen bonds, such as the anionic detergent sodium dodecyl sulfate (SDS) (Bietz and Wall 1972). Following these treatments the component subunits can be separated by electrophoresis in polyacrylamide gels containing SDS (SDS–PAGE). However, when SDS–PAGE has been used to examine the glutenin subunit composition of different wheat cultivars, varying results have been obtained in different laboratories. Thus Bietz et al. (1975) examined 80 cultivars of hexaploid bread wheat and found 75 of them to have glutenin subunit patterns which were identical or quite similar. By contrast, Orth and Bushuk (1973), who examined 26 cultivars of hexaploid wheat, detected more extensive variation in glutenin subunit patterns.

However, the resolution of protein subunits obtained in these studies was less than ideal because both groups of workers employed a single, continuous-pH gel system. Since discontinuous-pH, two-gel systems, which first concentrate the proteins into a narrow starting zone, generally give better resolution of protein bands than single-gel systems, we decided to use such a system to separate the glutenin subunits with the aim of determining more precisely the degree of variation that exists for glutenin subunits in cultivars of hexaploid wheat. This paper reports the results of using such a system to separate the total endosperm storage proteins of wheat. The good resolution obtained permitted extensive variation to be detected in the glutenin subunits of different wheat cultivars. An analysis of this variation is included in this paper.

Materials and Methods

The discontinuous system of SDS–PAGE used is based on the system of Laemmli (1970). Gels (145 by 100 by 2 mm) were poured as vertical slabs between two glass plates clamped to the side of a stand similar to that described by Studier (1973). The slot-forming comb (see Studier 1973) had prongs 4 mm wide spaced 4.5 mm apart. The separating gel contained 8.33% (w/v) acrylamide, 0.067% (w/v) bisacrylamide (Bis), 0.1% (w/v) SDS and 0.375 M tris(hydroxymethyl)aminomethane (Tris) made to pH 8.8 with HCl. The 13-mm stacking gel contained 3% (w/v) acrylamide, 0.08% (w/v) Bis, 0.1% (w/v) SDS and 0.125 M Tris made to pH 6.8 with HCl. Both gels were polymerized with N,N,N',N'-tetramethyl-ethylenediamine and ammonium persulfate. The electrode buffer (for both upper and lower tanks) contained 0.1% (w/v) SDS and 0.025 M Tris made to pH 8.3 with glycine.

To prepare the protein samples, wheat grains were cut into halves and the half with the embryo was discarded or saved to be grown into a plant. The other half was cut into four pieces which were placed in a small glass phial to which was added 0.14 ml of the extracting buffer prepared by freshly mixing 1 ml of a stock solution (0.125 M Tris, 8% (w/v) SDS, 20% (v/v) glycerol, 0.002% (w/v) bromophenol blue made to pH 6.8 with HCl) with 1 ml of water and 30 μl 2-ME. The phials were then corked and incubated overnight at approximately 40°C.

Prior to electrophoresis, sample tubes were centrifuged at 3000 rev/min for 10 min and 20 μl of the clear supernatant loaded into each well. Electrophoresis was carried out with the negative terminal in the upper tank and at a constant current (46 mA) for the first 30 min and then at 26 mA for the remainder of the run. The run was terminated when the marker dye reached the bottom of the gel (about 5 h). Gels were run at room temperature or in a refrigerator which was switched off during the run. Gels were stained overnight in a staining solution consisting of one part of 1% (w/v) Coomassie Brilliant Blue R mixed with 40 parts of 6% trichloroacetic acid in water, methanol and glacial acetic acid (80:20:7). Gels were destained for 24 h in water, methanol and glacial acetic acid (114:48:8) and then stored in plastic bags at 4°C.

The wheat cultivar Chinese Spring was included in all gels as a standard and the mobilities of the glutenin subunit bands in other wheat cultivars were scored relative to the mobilities of the Chinese Spring bands. This procedure made it unnecessary to compare patterns between gels, thereby avoiding any difficulties associated with slight differences between gels in mobility of bands.
Results

Chromosomal Control of Glutenin Subunits

To assist in interpreting the differences that were found in the glutenin subunit banding patterns of different cultivars, the chromosomal control of these subunits in the cultivar Chinese Spring will first be examined. The banding pattern obtained with crude extracts of the total endosperm storage proteins of Chinese Spring is shown in Fig. 1a, together with the banding pattern of supposedly pure glutenin protein extracted from flour made from Chinese Spring wheat (Fig. 1d). Comparison of these two patterns indicates that the four slowest moving bands in the Chinese Spring pattern (bands 1–4 in Fig. 1a) represent glutenin protein subunits. The glutenin extract (Fig. 1d) also contains some fast-moving protein species which are not well resolved. The present paper is restricted to an analysis of the variation that occurs in the well-resolved, slow-moving glutenin subunits.

As shown in Fig. 1, Chinese Spring aneuploids which lack chromosome 1D (nulli-1D) do not possess band 1 and have a lightly stained band 4, and stocks which lack chromosome 1B (nulli-1B) do not possess bands 2, 3, 5, 6 and 7 normally present in Chinese Spring. This observation indicates that band 1 and the principal component of band 4 are controlled by genes located on chromosome 1D and bands 2, 3, 5, 6 and 7 are controlled by genes located on chromosome 1B. Tests with stocks deficient for individual arms of chromosomes 1D and 1B have shown that bands 1 and 4 are controlled by genes on the long arm of 1D, bands 2 and 3 by genes on the long arm of 1B, and bands 5, 6 and 7 (which are considered to be
gliadins rather than glutenin subunits) by genes on the short arm of 1B. Thus, in Chinese Spring, the slow-moving glutenin subunits (bands 1–4 in Fig. 1) are controlled by genes on the long arms of chromosomes 1D and 1B. This conclusion is in agreement with previous findings (Bietz et al. 1975). The molecular weights of bands 1, 2, 3 and 4 in Chinese Spring have been estimated to be 133000, 104000, 93000 and 86000 respectively (Bietz et al. 1975).

The glutenin protein used as a reference sample (see Fig. 1) was prepared by sequential extraction of flour: twice with 0·04 M NaCl, twice with 70% ethanol and finally 0·7% acetic acid. The glutenin protein solubilized by the latter solvent was precipitated by adding ethanol to make the solution 70% with respect to ethanol and then adjusting to pH 6·6–8·0 with NaOH (see Bietz et al. 1975). The precipitation step removed traces of contaminating gliadins not removed by the previous treatments. Although this glutenin protein extract gave qualitatively the same banding pattern for the high molecular weight glutenin subunits as the protein extracted directly from the grain, there was an unexplained difference in the relative staining intensity of bands with these two methods (cf. bands 3 and 4 in Figs 1a and 1d).

**Variation in Glutenin Subunit Banding Patterns of Hexaploid Wheat**

The variation that occurs in the glutenin subunit banding patterns of hexaploid wheat cultivars was examined by testing 98 wheat cultivars collected from around the world and maintained at the Waite Agricultural Research Institute. One of the gels is shown in Fig. 2 in which it can be seen that much variation occurs in the glutenin subunit banding patterns as well as in the pattern of the faster moving bands. The extent of this variation gives a high probability that any two cultivars chosen at random will possess different banding patterns. Therefore SDS–PAGE of wheat endosperm proteins provides a useful means, in addition to those already available, of identifying wheat cultivars.

An examination of the glutenin subunit banding patterns of the 98 cultivars revealed that some bands, or band combinations, never occur together in the same cultivar, i.e. some bands, or band combinations, behave as alternatives to each other. Three groups of such bands were identified and these are shown in Fig. 3, together with the frequency with which each alternative occurred. The different glutenin subunit banding patterns can be accounted for by assuming that each cultivar possesses one of the alternative forms from each of the three groups.

There is evidence that the alternative bands, or band combinations, within each group are controlled by genes on the same chromosome. Thus, in group 1 in Fig. 3, two pairs of bands occur as alternatives. Pattern a corresponds to Chinese Spring bands 1 and 4 and in Chinese Spring these bands are controlled by genes on chromosome 1D. Evidence that the genes controlling the two bands in pattern b also are located on chromosome 1D comes from testing a Chinese Spring–Hope 1D substitution line. This stock possesses chromosome 1D of the cultivar Hope in a near-Chinese Spring background. Hope possesses pattern b and the Chinese Spring–Hope 1D substitution line possesses the Hope pattern b instead of the Chinese Spring pattern a, as expected if pattern b is controlled also by chromosome 1D in Hope (Fig. 4e). There is indirect evidence that the genes on chromosome 1D controlling the two bands of patterns a and b are closely linked. If they were loosely linked, it would be expected that they would have recombined at some stage in the
past, thereby generating two new patterns in wheat; namely, a case in which the slow-moving band of pattern \( a \) (Fig. 3) was associated with the fast-moving band of pattern \( b \) and a case in which the slow-moving band of pattern \( b \) was associated with the fast-moving band of pattern \( a \). Since examples of these latter band combinations have not been found so far, recombination between the genes is apparently rare, thus indicating that the genes are probably closely linked.

![Figure 2: SDS-PAGE patterns of total endosperm storage proteins of hexaploid wheat cultivars.](image)

(a) Jaral *S* */Lee-sk-mana; (b) Vicam-71; (c) Potam-70; (d) Chinese Spring; (e) Sonalika; (f) Chhoti Lerma; (g) Piamontes; (h) Pato Argentino; (i) Chinese Spring; (j) Zambezi; (k) Xelaju-66; (l) Kalyansona-227; (m) Huelquen.

In group 2 in Fig. 3, three different patterns occur, one of which, pattern \( c \), is a null form. All cultivars possess either a band of slightly greater mobility than Chinese Spring band 1 (Fig. 3, pattern \( d \)) or a band of slightly less mobility (Fig. 3, pattern \( e \)), or, as in Chinese Spring, neither of these bands. The genes controlling the single bands of patterns \( d \) and \( e \) are apparently located on chromosome 1A of wheat, since the cultivar Cheyenne possesses pattern \( d \) and this band is added to the Chinese Spring pattern in the Chinese Spring–Cheyenne 1A substitution line and Hope possesses pattern \( e \) and this band is added to the Chinese Spring pattern in the Chinese Spring–Hope 1A substitution line (see Fig. 4c). At present it is not known whether the gene or genes which control these bands are located on the long or short arm of chromosome 1A.

Finally, considering the different patterns in group 3 (Fig. 3), pattern \( l \) corresponds to Chinese Spring bands 2 and 3 which are controlled by chromosome 1B in
Chinese Spring. Hope possesses pattern \( m \) and in Chinese Spring–Hope 1B substitution line pattern \( l \) is replaced by pattern \( m \) (see Fig. 4d). In addition Chinese Spring bands 5, 6 and 7 which are controlled by genes on the short arm of chromosome 1B also are replaced in the substitution line by the Hope pattern in this

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\begin{align*}
\text{Group 1} & \quad \text{Group 2} & \quad \text{Group 3} \\
4 & \quad & \\
3 & \quad & \\
2 & \quad & \\
1 & \quad & \\
\end{align*}
\]

\[\text{CS} \quad a \quad b \quad c \quad d \quad e \quad f \quad g \quad h \quad i \quad j \quad k \quad l \quad m \quad n\]

Fig. 3. Diagram showing the three groups of glutenin subunit bands, or band combinations, where the bands, or band combinations, within each group are alternatives to each other. Chinese Spring bands 1–4 are shown for comparison. Amongst a world collection of 98 wheat cultivars the number of cultivars possessing each band, or band combination, was as follows—Group 1: \( a = 43, \ b = 54 \); Group 2: \( c = 24, \ d = 37, \ e = 37 \); Group 3: \( f + n = 23, \ g = 5, \ h = 13, \ i = 12, \ j = 7, \ k = 2, \ l = 29, \ m = 7 \).

![SDS-PAGE patterns of total endosperm storage proteins of wheat cultivar Hope (a), Chinese Spring (b), Chinese Spring-Hope 1A substitution (c), Chinese Spring-Hope 1B substitution (d), Chinese Spring-Hope 1D substitution (e).](image)

Fig. 4. SDS-PAGE patterns of total endosperm storage proteins of wheat cultivar Hope (a), Chinese Spring (b), Chinese Spring-Hope 1A substitution (c), Chinese Spring-Hope 1B substitution (d), Chinese Spring-Hope 1D substitution (e).

section of the gel. Chinese Spring–Timstein 1B and Chinese Spring–Cheyenne 1B substitution lines also have been tested and in these the Chinese Spring pattern (Fig. 3, pattern \( l \)) is replaced by patterns \( i \) and \( n \) respectively. Thus, of the nine different patterns allocated to group 3 (Fig. 3) there is direct evidence that four of
them—\(i, \, l, \, m\) and \(n\)—are controlled by a gene or genes on chromosome 1B. It would seem likely that the remaining five patterns in this group also are controlled by genes on chromosome 1B, but direct evidence for this has not yet been obtained.

Three further points may be noted concerning the different patterns in group 3 (Fig. 3). First, the single band of pattern \(h\) (see Fig. 3) appears on gels as an unusually broad band. However, when gels are run for longer periods (greater than 5 h) this broad band is resolved into two separate bands: a strongly staining band and a weaker band of slightly slower mobility. The second point to be noted is that patterns \(f\) and \(n\) in group 3 cannot always be distinguished from each other. The single band of pattern \(f\) has the same migration rate as the slowest moving band in pattern \(n\). Pattern \(n\) is distinguished by the presence of a second, faster moving band. However, this band has the same migration rate as the fastest moving band of pattern \(b\) in group 1. Therefore patterns \(f\) and \(n\) cannot be distinguished in a banding pattern which possesses pattern \(b\) of group 1. For this reason the combined frequency of patterns \(f\) and \(n\), and not the individual frequencies, has been given in the caption to Fig. 3. The third point to be noted is that some of the cultivars possessing pattern \(j\) also possess a weakly staining band of slightly faster mobility than band 3 in Chinese Spring (see Fig. 3). This band was not included in Fig. 3 because in some cultivars it was so faint that it was barely evident.

So far three wheat cultivars have been found with banding patterns which differ from those outlined above. The cultivar BT-2288 from Tunisia has a prominent band of slightly greater mobility than the slowest moving band of pattern \(b\) (Fig. 3) which has not been found in any other cultivar. The chromosome which controls this band is unknown, but since BT-2288 does not possess either of the bands known to be controlled by chromosome 1A (patterns \(d\) and \(e\) in Fig. 3), the exceptional band may be controlled by chromosome 1A. The cultivar Chanab-70 from Pakistan appears to possess a rare group 1 pattern. This cultivar has a band with the same mobility as the slowest moving band of pattern \(a\) (Fig. 3), but does not have a band with the same mobility as the second band in pattern \(a\). Instead, it has a band with marginally faster mobility than the second band in pattern \(a\). The remaining cultivar with an exceptional pattern is Nap Hal which does not possess either pattern \(a\) or pattern \(b\) in group 1 (Fig. 3); this cultivar possesses only two slow-moving glutenin subunits which correspond to pattern \(l\) in group 3 (Fig. 3). This unusual pattern of Nap Hal has been noted before (Bietz et al. 1975). Apparently therefore the genes on chromosome 1D which control the glutenin subunits are either missing in Nap Hal (due to a deletion) or, for some reason, are not expressed. Nap Hal appears to possess two reciprocal chromosome translocations since two quadrivalents were observed at metaphase I of meiosis in pollen mother cells of an \(F_1\) plant derived from crossing Nap Hal to the Australian cultivar Gabo. However, it is not known which chromosomes are involved in these translocations, or whether the translocations may be related to the unusual glutenin composition of Nap Hal.

\textit{Variation in Banding Patterns in Aegilops squarrosa}

Since the \(D\) genome of hexaploid wheat (\(AABBDD\)) is known to have come from \textit{Aegilops squarrosa} (\(DD\)) (Kihara 1944; McFadden and Sears 1946) 28 \textit{A. squarrosa} accessions were tested to see if any possessed a pair of slow-moving bands which matched those of either pattern \(a\) or pattern \(b\) in group 1 (Fig. 3) which are controlled by chromosome 1D in wheat. All 28 accessions possessed a pair of bands
with similar mobility to the wheat glutenin subunits, and eight different banding patterns were identified. Examples of these eight different patterns are shown in Fig. 5, together with two wheat cultivars (samples a and d in Fig. 5) possessing the two 1D patterns for comparison. As shown in Fig. 5, two A. squarrosa accessions (samples b and c) have band patterns which come close to matching the 1D bands of wheat sample a and two other A. squarrosa accessions (samples e and f) have patterns which come close to matching the 1D bands of wheat sample d.

![SDS-PAGE patterns of total endosperm storage proteins of two wheat cultivars possessing the alternative banding patterns controlled by chromosome 1D (samples a and d) and 10 A. squarrosa accessions (samples b, c and e-l). Wheat cultivars: (a) Chinese Spring, (d) Chinese Spring–Hope 1D substitution. A. squarrosa variety (number in brackets following each variety is Waite Agricultural Research Institute accession number): (b) typica (7503), (c) strangulata (7505), (e) meyeri (7302), (f) strangulata (7022), (g) typica (7494), (h) typica (7506), (i) strangulata (7301), (j) strangulata (7298), (k) strangulata (7297), (l) strangulata (7504).](image)

From this limited survey it seems that only a small proportion of the variation in glutenin subunits that is present in A. squarrosa is present in hexaploid wheat. This may indicate that the present-day hexaploid wheats are derived from just a few (perhaps only two) A. squarrosa stocks. However, the possibility cannot be excluded that some of the variation initially introduced into hexaploid wheat from A. squarrosa has subsequently been lost, either by chance or selection.

**Discussion**

**Relationships between Storage Proteins in Wheat**

The finding that the slow-moving glutenin subunits of wheat are controlled by genes on chromosomes 1A, 1B and 1D is of interest because Shepherd (1968) has
shown that these chromosomes also possess genes controlling gliadin proteins. The possibility that the same protein species constitute both the gliadin proteins and the glutenin subunits can be rejected however, because in Chinese Spring the genes on chromosomes 1A, 1B and 1D controlling gliadin proteins are located on the short arms of these chromosomes (Shepherd 1968) whereas the glutenin subunits are controlled by genes on the long arms of chromosomes 1B and 1D (in the case of chromosome 1A, the arm location of the gene or genes controlling the glutenin subunit or subunits has not yet been determined). If it is assumed that chromosomes 1A, 1B and 1D are each derived from a common ancestral chromosome, then it is likely that the glutenin protein subunits controlled by each of these chromosomes are derived from the same ancestral protein species. If this is the case, then the observation that the patterns in SDS gels of the glutenin subunits controlled by each of the chromosomes 1A, 1B and 1D are distinctly different from each other (see Fig. 3, groups 2, 3 and 1, respectively) suggests that these protein species have undergone considerable changes since their isolation into separate genomes. Furthermore, the observation that chromosomes 1B and 1D each control at least two glutenin subunit bands whereas chromosome 1A controls only a single band also suggests that gene duplication or gene loss may have occurred.

Nature of Differences between Glutenin Subunits

As indicated above, an important question arising out of the present study concerns the nature of the differences between the glutenin subunits which cause them to migrate at different rates in SDS–PAGE. Protein species treated with SDS and 2-ME and then electrophoresed in gels containing SDS are usually separated by polypeptide chain-length differences only, since a linear relationship has been found to exist between the logarithm of polypeptide molecular weight and electrophoretic mobility for most protein species (e.g. Dunker and Rueckert 1969; Weber and Osborn 1969).

In the present study of wheat glutenin subunits there are several instances where glutenin subunits with different mobilities in SDS gels, and which therefore may have different chain lengths, occur as alternatives to each other. Since the simplest explanation for protein species behaving as alternatives is that they are controlled by alternative forms of the same gene (alleles), the possibility must be considered that alleles controlling glutenin subunits may be coding for polypeptides of different lengths.

The apparent occurrence of extensive heterogeneity in the size of protein units is not unique to the glutenin protein subunits but, as emphasized by Thomson and Doll (1979), appears to be common amongst storage protein polypeptides, at least in Triticum, Hordeum and Pisum species. Thomson and Doll point to three mechanisms which might give rise to such heterogeneity. One possibility comes from the finding that the transcriptional unit of the eukaryotic genome may contain intervening sequences which are excised prior to translation, so that, as noted by Gilbert (1978), a single base change at the boundary of a region to be spliced out could change the splicing pattern, thereby resulting in the deletion or addition of whole sequences of amino acids. A second possibility is that post-translational cleavage of a precursor might introduce heterogeneity in size if variation occurred in the location of the cleavage point, since there is evidence that some storage proteins in maize (zeins) are synthesized as precursors from which a short sequence
of amino acids is subsequently removed by proteolytic cleavage (see Larkins and Hurkman 1978). A third possibility is that an attached moiety (e.g. carbohydrate) is responsible for the different mobilities: the moiety attached in one cultivar may be different from that attached in another, or the differences may be caused by the presence or absence of an attached moiety. In addition to these three possible mechanisms, another possibility is suggested by recent studies of the gene in silkworms coding for silk fibroin protein (Sprague et al. 1979). This protein has a repeated amino acid sequence coded for by a repeated DNA sequence. Because of this repeated DNA sequence, unequal crossing-over has apparently occurred in the past, giving rise to allelic genes of unequal size which produce fibroin molecules of different size. If storage protein genes contain a repeated DNA sequence (or sequences) then alleles controlling polypeptides of different sizes also could have arisen by unequal crossing-over.

These mechanisms must remain speculative until critical evidence is obtained from further studies, such as direct molecular weight measurements, peptide digests or, ideally, amino acid sequencing of particular protein subunits. If it is found that differences in molecular size are not responsible for the observed differences in mobility in SDS gels of bands which behave as alternatives, then an explanation would have to be sought in amino acid differences which cause the subunits to assume different shapes in SDS solutions or to bind different amounts of SDS, or which affect the arrangement of the bound SDS molecules. The possibility that amino acid substitutions are responsible for the mobility differences cannot be rejected because two recent studies of mammalian α-crystallin A chains (molecular weight 20000) and a histidine-transport protein (molecular weight 25000) have shown that some single amino acid substitutions in these proteins can cause mobility differences in SDS gels corresponding to an apparent molecular weight change of 1000 to 2000 (De Jong et al. 1978; Noel et al. 1979).

Although there is, as yet, no direct information available concerning the molecular basis for the mobility differences in SDS gels of glutenin subunits which behave as alternatives, there is one observation which may provide some indirect evidence regarding this problem. This observation relates to the two alternative banding patterns controlled by chromosome 1D in wheat (Fig. 3, group 1) and to the patterns of the equivalent bands in A. squarrosa (Fig. 5).

As shown in Figs 3 and 5, each of the patterns under consideration consists of a pair of bands. In Fig. 5 the different banding patterns found in A. squarrosa accessions have been arranged in order of the distance apart of the two bands. An examination of these banding patterns indicates that, overall, there is an apparent negative correlation between the mobilities of the two bands; for example, progressing from sample e through to sample l in Fig. 5, the band nearest the origin tends to decline in mobility whereas the second band tends to increase in mobility. If these protein species are being separated by size differences only, then the apparent negative correlation between the mobility of the two bands suggests that as one protein species increases in size, the other tends to decrease in size.

Three mechanisms can be envisaged which would give rise to a relationship of this kind. One possibility is that the two protein species are derived from a larger molecule that is cut into two and that the larger molecule is polymorphic with respect to the position at which the cut occurs. It would follow from this that as one fragment increased in size due to a change in the location of the cut, the other
would decrease in size. A second possibility is that the two protein molecules are derived from one mRNA molecule which is cut into two parts prior to translation into polypeptide chains; it is known that mRNA molecules in eukaryotes are sometimes cut prior to translation in order to remove intervening sequences (Tilghman et al. 1978). If variation existed in the location of the cut point, then one fragment would decrease in size as the other increased in size. A third possibility is that the DNA segments coding for the bands are adjacent to each other, with the point separating the segments occurring at different places in different wheat cultivars or A. squarrosa accessions. Again, as the point separating the two segments altered so as to increase the size of one segment, the other would decrease in size. It is a requirement of each of these mechanisms that the DNA segments coding for the two protein species be adjacent to each other. This requirement is supported by the argument advanced earlier that in wheat the DNA segments on chromosome 1D are apparently closely linked because the banding patterns expected to arise from recombination of the segments have not been found so far.

It is possible that much more variation exists in the glutenin protein subunits than has been identified in the present study, since, if the variation detected in the present study is due to chain-length differences only, protein bands with identical mobilities need not possess identical amino acid sequences. Therefore two kinds of variation may occur in the glutenin subunit coded for by a particular locus. Firstly, the length of the protein species may vary and, secondly, protein species of the same length may have amino acid differences. Thus loci controlling glutenin subunits may possess alleles controlling differences in both size and amino acid constitution.

A question arising out of the present study is whether differences in composition of glutenin subunits between cultivars as revealed by SDS gel electrophoresis may be related to differences in dough properties or baking quality of flour. To explore the possibility of such a relationship an examination was carried out of the glutenin subunit banding patterns of 23 Australian wheat cultivars which Moss (1973) has listed in order of their gluten stability, a characteristic which is an expression of the quality of the protein and an important flour property since it influences the extensibility of dough. However, no relationship was evident between glutenin subunit banding pattern and gluten stability.

In conclusion, the present study provides indirect evidence that glutenin subunits, apparently controlled by different alleles of the same locus, may vary in polypeptide chain length. Therefore this study adds to previous observations that heterogeneity for size may be characteristic of seed storage proteins in wheat. Further, by more precisely identifying the high molecular weight glutenin subunits, and their genetic control, the results of the present study should enable future studies of these subunits to be carried out with greater precision.

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