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Fractionation of Wheat Gliadin and Glutenin Subunits by Two-dimensional Electrophoresis and the Role of Group 6 and Group 2 Chromosomes in Gliadin Synthesis

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Summary. Subunits of wheat endosperm proteins have been fractionated by two-dimensional electrophoresis. To determine which subunits in the two-dimensional electrophoretic pattern belong to gliadin or glutenin the endosperm proteins have also been fractionated by a modified Osborne procedure and by gel filtration on Sephadex G-100 and Sepharose CL-4B prior to separation by two-dimensional electrophoresis.

The control of production of five major grain protein subunits is shown to be determined by chromosomes 6A, 6B and 6D by comparing two-dimensional electrophoretic protein subunit patterns of aneuploid lines of the variety 'Chinese Spring'. From these and previous studies it is concluded that some α, β and γ gliadins (molecular weights by SDS-PAGE 30,000 to 40,000) are specified by genes on the short arms of homoeologous Group 6 chromosomes, the ω gliadins (molecular weights by SDS-PAGE 50,000 to 70,000) are specified by genes on the short arms of homoeologous Group 1 chromosomes and the glutenin subunits (molecular weights by SDS-PAGE > 85,000) are specified by genes on the long arms of homoeologous Group 1 chromosomes.

No major gliadins or glutenin subunits were absent when any of the chromosomes in homoeologous Groups 2, 3, 4, 5 or 7 were deleted. However two gliadins whose presumed structural genes are on chromosome 6D were absent in aneuploid stocks of 'Chinese Spring' carrying two additional doses of chromosome 2A. Two out of thirty-three intervarietal or interspecific chromosome substitution lines examined, involving homoeologous Group 2 chromosomes, lacked the same two gliadins. All the subunits in the other thirty-one chromosome substitution lines were indistinguishable from those in 'Chinese Spring'. It is therefore concluded that the major variation affecting gliadin and glutenins in wheat is concentrated on the chromosomes of homoeologous Groups 1 and 6 but Group 2 chromosomes are candidates for further study.

An endosperm protein controlled by chromosome 4D in 'Chinese Spring' is shown to be a high molecular weight globulin.

Key-words: *Triticum aestivum* – Glutenin – Gliadin – Electrophoresis

Introduction

The major endosperm proteins of wheat (*Triticum aestivum*) have received considerable attention in research because of their importance in nutrition and bread-making. In early studies of the endosperm proteins of wheat, Osborne (1907) fractionated the proteins on the basis of their solubility in different solvents. The four solubility classes are albumins, soluble in water; globulins, soluble in 10% NaCl; gliadin, soluble in 70% ethanol; and glutenin, soluble in acid or alkali solutions.

The gliadin and glutenin fractions comprise most of the protein in the wheat grain (Huebner and Wall 1976). The component polypeptides of gliadin fractions were separated in a gel electrophoretic system in a pH 3.1 aluminium lactate buffer by Jones et al. (1959). Woychik et al. (1961) proposed the classification of the polypeptides into α, β, γ, and ω gliadins on the basis of their mobility in this aluminium lactate buffer system. The components of the α, β, γ and ω classes of gliadins have been resolved and purified by gel filtration chromatography (Charbonnier 1974; Bietz and Wall 1972; Bietz et al. 1977; Payne and Corfield 1979). Most of the α, β and γ gliadins have molecular weights of 36,000 to 44,000 (Bietz and Wall 1972) while those of the ω gliadins lie between 65,000 and 79,000 (Booth and Ewart 1969; Bietz and Wall 1972; Charbonnier 1973, 1974) as determined by SDS polyacrylamide gel electrophoresis (SDS-PAGE). These molecular weight estimates, particularly those for ω gliadins are over-estimates, owing to the unusual behaviour of these proteins in SDS-PAGE (Sexson et al. 1978; Hamauzu et al. 1974, 1975). Another gliadin fraction called 'high molecular weight gliadin' has been characterized as the fraction which appears in the void volume when the ethanol-soluble fraction extracted from gluten is chromatographed on Sephadex

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G-100 (Beckwith et al. 1966). The molecular weight of this fraction is approximately 100,000 and appears to be composed mainly of subunits of 34,500 and 44,000 after reduction, as determined by SDS gel electrophoresis (Bietz and Wall 1973; Payne and Corfield 1979).

Glutenin has been fractionated by gel filtration in Sepharose 4B (Huebner and Wall 1976; Payne and Corfield 1979). The resulting fractions contain aggregates of very high molecular weights. On electrophoresis in sodium dodecyl sulphate these aggregates break down into components of apparent molecular weights varying from approximately 150,000 to values similar to those of α, β, and ω gliadins (Bietz and Wall 1972). The large aggregates of glutenin are probably linked by intermolecular disulphide bonds (Nielson et al. 1962; Woychik et al. 1964) and/or numerous secondary bonding forces: hydrogen bonding, ionic bonding, and hydrophobic interactions (Bernardin and Kasarda 1973).

Many studies of wheat storage proteins have centred on the genetic control of these gliadin and glutenin components. Most studies have made use of the aneuploid series in the wheat variety 'Chinese Spring' (Sears 1954). From comparisons of electrophoretic patterns of grain proteins from various aneuploids, in particular nullisomic-tetrasomic and ditelosomic lines (Sears 1954), the presumed structural genes of a number of wheat grain proteins have been allocated to certain chromosomes or chromosome arms.

One-dimensional electrophoretic techniques have enabled the control of many storage proteins to be allocated to the chromosomes of homoeologous Groups 1 and 6 (Shepherd 1968; Bietz et al. 1975). These results were extended by the two-dimensional system of Wrigley and Shepherd (1973) which combined isoelectricfocusing with starch gel electrophoresis in aluminium lactate buffer. Using a two-dimensional technique based on that of O'Farrell (1975), which combines isoelectricfocusing with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), we have previously described the control of 14 of the 29 major endosperm protein subunits of 'Chinese Spring' by chromosomes 1A, 1B, and 1D (Brown et al. 1979). In order to relate these subunits to those classified differently by other workers (Woychik et al. 1961; Beckwith et al. 1966; Beitz and Wall 1972; Charbonnier 1973; Huebner and Wall 1976; Payne and Corfield 1979) we have now analysed by two-dimensional SDS-PAGE, 'Chinese Spring' storage proteins fractionated by a modified Osborne procedure and by gel filtration.

There are few reports on the regulation of the production of grain proteins by chromosomes other than those presumed to carry the structural genes. However, the chromosomes of Group 2 have been implicated in the control of total grain protein content from studies on intervarietal and interspecific chromosome substitution lines (Law et al. 1978). Shepherd (1968) reported that four doses of chromosome 2A in the variety 'Chinese Spring' seemed to suppress one gliadin component and promote the presence of another. Waines (1973) has also suggested that the chromosomes of Group 2 of 'Chinese Spring' may have a regulatory effect on gliadin levels from electrophoretic studies.

At least one major grain protein appears to be specified by chromosome 4D because in two lines, nullisomic 4D tetrasomic 4A and nullisomic 4D tetrasomic 4B a 68,000 molecular weight component is absent (Bietz et al. 1975).

The roles of homoeologous Group 6 chromosomes, Group 2 chromosomes, and certain Group 4 chromosomes in determining grain protein composition in wheat have therefore been investigated by two-dimensional electrophoresis.

Materials and Methods

Genetic Stocks

Fractionation studies were carried out using grain of the wheat variety 'Chinese Spring'. Genetic studies made use of the aneuploid lines in the genetic background of 'Chinese Spring' which were developed by Sears (1954) and have been maintained at the Plant Breeding Institute for many years. The lines used are of four types: nullisomic-tetrasomic, tetrasomic, ditelosomic, and chromosome substitution lines. A nullisomic-tetrasomic line has a pair of chromosomes deleted but replaced by an extra pair of homoeologous chromosomes. A tetrasomic line has an extra pair of homologous chromosomes, and a ditelosomic line has a chromosome arm of each of a pair of chromosomes missing. A substitution line has a pair of 'Chinese Spring' chromosomes deleted and replaced by the homologous pair from another variety (intervarietal chromosome substitution) or by a homoeologous pair from another related species (interspecific chromosome substitution). The lines used are described as follows:

1. 'Chinese Spring' (CS) Euploid
2. Aneuploids

CS N2A T2B (Nullisomic 2A-tetrasomic 2B)	CS N6A T6B
CS N2A T2D	CS N6A T6D
CS N2B T2A	CS N6B T6A
CS N2B T2D	CS N6D T6A
CS N2D T2A	CS N6D T6B
CS N2D T2B	CS T6A
CS T2A (Tetrasomic 2A)	CS T6B
CS T2B	CS T6D
CS T2D	CS DT6A^{s} (Ditelosomic: long arms missing)
CS N4D T4A	CS DT6B^{s}
CS N4D T4B	CS DT6Dα
	CS DT6Dβ

3. Chromosome substitution lines

a) Lines in which chromosomes 2A, 2B and 2D of 'Chinese Spring' have been replaced by their homologues from the varieties 'Cappelle-Desprez', 'Cheyenne', 'Ciano 67', 'Hope', 'Lutescens 62', 'Synthetic', 'Timstein', and *T. spelta*. These lines were developed by C.N. Law and his colleagues at this Institute with the exception of those containing 'Hope' and 'Timstein' chromosomes, which were obtained from E.R. Sears, University of Missouri, and those containing 'Cheyenne' chromosomes, which were obtained from R. Morris, University of Nebraska.

b) Interspecific chromosome substitution lines in which chromosomes 2M from *Aegilops comosa* (Riley et al. 1968), 2C^{u} from *Ae. umbellulata*, and 2R^{m} from *Secale montanum* have been substituted for chromosomes 2A, 2B and 2D of 'Chinese Spring'. The *Ae. umbellulata* and *S. montanum*

substitution lines were derived by V. Chapman and T.E. Miller of this Institute.

Fractionation of Grain Proteins by a Modified Osborne Sequential Extraction

Wheat storage proteins were fractionated by the modified Osborne procedure of Chen and Bushuk (1970). The four fractions obtained: albumin, globulin, gliadin, and glutenin, were dissolved in 2M urea, 0.5% SDS, and 0.6% β-mercaptoethanol prior to analysis by two-dimensional electrophoresis.

Extraction of Gliadin and Fractionation by Sephadex G-100 Gel Filtration

A 70% (v/v) ethanol-soluble gliadin fraction was extracted from 20 g of 'Chinese Spring' euploid flour, prepared for and fractionated by gel filtration on a Sephadex G-100 column, as described by Payne and Corfield (1979). Prior to two-dimensional electrophoresis, the lyophilized, pooled fractions were taken up in 2M urea, 0.5% (w/v) SDS, and 0.6% β-mercaptoethanol.

Extraction and Fractionation of Glutenin by Sepharose CL-4B Gel Filtration

Protein was extracted and fractionated on a Sepharose CL-4B gel filtration column using the method of Payne and Corfield (1979). The freeze-dried, pooled fractions were prepared for electrophoresis as described above.

Grain Protein Extraction and Electrophoresis

Protein from the grain of 'Chinese Spring' and its aneuploids was extracted and analysed by two-dimensional electrophoresis as described previously (Brown et al. 1979; Brown et al. 1981). Molecular weight determinations are also described in Brown et al. (1979). The reproducibility of the protein patterns and the usefulness of this two-dimensional system have been discussed previously (Brown et al. 1979).

Results

Subunit Analysis of Proteins Fractionated by a Modified Osborne Sequential Extraction

Flour from the variety 'Chinese Spring' was fractionated by the modified Osborne system of Chen and Bushuk (1970). The two-dimensional subunit patterns of the four fractions obtained: albumin, globulin, gliadin, and glutenin are shown in Figure 1b-1e respectively. The subunit pattern of 'Total' protein, extracted from flour in 2 M urea, 0.5% SDS, and 0.6% β-mercaptoethanol, is shown in Figure 1a. The subunits are shown more clearly and numbered for identification purposes in Figure 2. Both the albumin and the globulin extracts contain high molecular weight subunits in addition to the expected low molecular weight albumins and globulins. The high molecular weight proteins (58,000 to 72,000) do not form specific spots but 'streak' across the gel. These proteins differ between the albumin and globulin fractions, and there are no such proteins in the gliadin and glutenin extracts (Fig. 1d and 1e).

The two-dimensional subunit pattern of the 70% ethanol gliadin extract (Fig. 1d) clearly includes subunits 7-8, 13-24, and small amounts of subunits 3-5 (Fig. 2). The 0.05 M acetic acid extracts (Fig. 1e) includes most of the 29 subunits numbered in Figure 2. The presence in the acetic acid extract of many of the subunits also found in the 70% ethanol extract suggests that the two 2-hour extractions in 70% ethanol were insufficient to extract all of subunits 13-24 and/or that these subunits are partly organised in protein complexes insoluble in 70% ethanol.

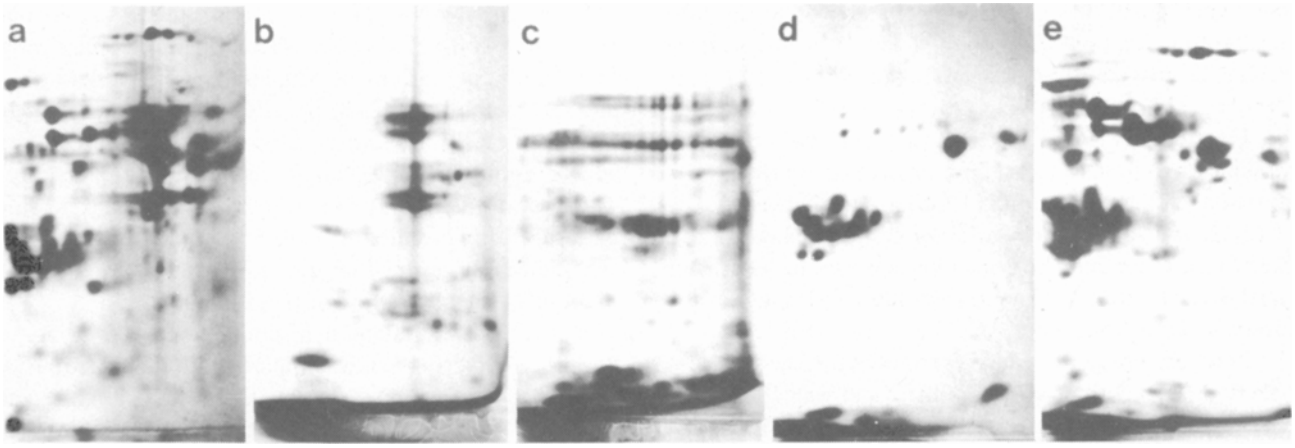

Fig. 1a-e. Two-dimensional protein subunit patterns of extracts of 'Chinese Spring' sequentially extracted by a modified Osborne technique. **a** 'Chinese Spring' 'total' extract (2 M urea, 0.5% SDS, 0.6% β-mercaptoethanol); **b** H_2O-soluble protein; **c** 1 M NaCl-soluble protein; **d** 70% ethanol-soluble protein; **e** 0.05 M acetic acid-soluble protein. 1200 μg protein per gel

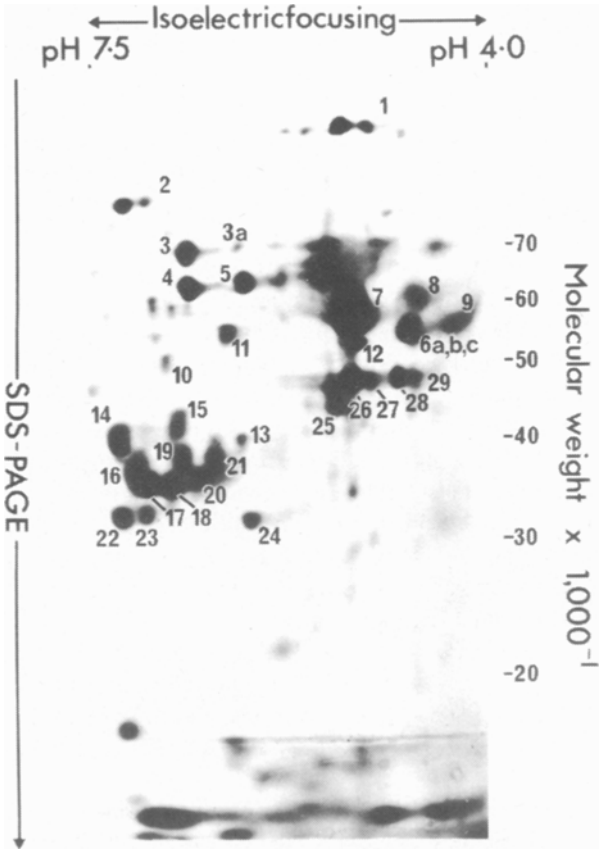

Fig. 2. Two-dimensional protein subunit pattern of 'Chinese Spring' euploid. 1200 μg protein loaded

Subunit Analysis of Gliadin fractionated by Sephadex G-100 Chromatography

The two-dimensional protein subunit pattern of the 70% ethanol gliadin extract (i.e. before fractionation) is shown in Figure 3a. Subunits 3-8, 12, and 13-24 are present. The two-dimensional subunit patterns of the proteins in three of the four fractions A, B and C (Fig. 3) recovered from Sephadex G-100 chromatography are shown in Figure 3b-d.

Fraction A (Fig. 3b) which eluted with the void volume corresponds to the high molecular weight gliadin fraction of Beckwith et al. (1966) and contains subunits 3-6, 12, and some members in the group numbered 13 to 23 (Fig. 2). Subunit 9 has also been shown to be present in this fraction on a gel with an extended pH range at the acidic end (gel not shown).

Fraction B (Fig. 3c) which corresponds to the ω-gliadin fraction (Beckwith et al. 1966; Bietz and Wall 1972) consists predominantly of subunits 3 to 8 and 12. Most or all of the subunits in low amounts with the possible exception of 17 and 19 (Fig. 2) could be due to incomplete separation of fractions A, B and C on Sephadex G-100.

Fraction C (Fig. 3d) represents a mixture of α, β and γ gliadins (Beckwith et al. 1966; Bietz and Wall 1972) and its two-dimensional subunit pattern is composed of subunits 13-24 with only trace amounts of subunits 3-5 (Fig. 2) which again could be contaminants due to incomplete separation on Sephadex G-100.

Subunit Analysis of Glutenin from a Total Protein Extract

Gel filtration of a total protein extract on a Sepharose CL-4B column generated four fractions (Fig. 4). Fractions I and II correspond to the glutenin I and II peaks of Huebner and Wall (1976) which contain proteins of molecular weights of up to 5,000,000 and between 100,000 and 5,000,000 respectively. The two-dimensional protein subunit pattern of Fraction I (Fig. 4b) after reduction consists of subunits 1 and 2 and traces of subunits 13, 14, 16-19 and 22 (Fig. 2). Fraction II (Fig. 4c) which represents a mixture of glutenin and ω-gliadin subunits, contains subunits 1-7 and 12 and traces of subunits 14, 16, 17, 19 and 22 (Fig. 2). Fraction III (Fig. 4d) corresponds to α, β and γ gliadin subunits and consists mainly of subunits 13-24 (Fig. 2).

The Control of Grain Proteins by Group 6 Chromosomes

The control of at least five distinct major protein subunits of different molecular weights and isoelectric points has been allocated to chromosomes 6A, 6B and 6D by using the nullisomic-tetrasomic and ditelosomic lines of Group 6. Correlation between the absence of a protein subunit in the two-dimensional pattern and the absence of a chromosome or chromosome arm from the genotype suggests that the particular chromosome controls the production of the subunit(s) in question.

When the two-dimensional protein subunit patterns of either N6AT6B (Fig. 5b) or N6AT6D (Fig. 5c) are compared with the pattern of 'Chinese Spring' euploid (Fig. 5a), two subunits (Fig. 2, nos. 20, 24) are absent in the aneuploid stocks. The molecular weights of these subunits appear approximately 35,000 and 32,000 respectively. These subunits are present in the two-dimensional subunit pattern of ditelosomic $6A^s$ (Fig. 5d) (where the long arms are deleted) suggesting that the short arms of chromosome 6A control the production of these subunits.

When the two-dimensional subunit pattern of 'Chinese Spring' euploid (Fig. 6a) is compared with that of N6BT6A (Fig. 6b), one subunit (Fig. 2, no. 14) with a molecular weight of 40,000 is deleted with the deletion of chromosome 6B. It is present in the subunit pattern of ditelosomic $6B^s$ (Fig. 6c) suggesting that the control of its production lies with the short arm of chromosome 6B.

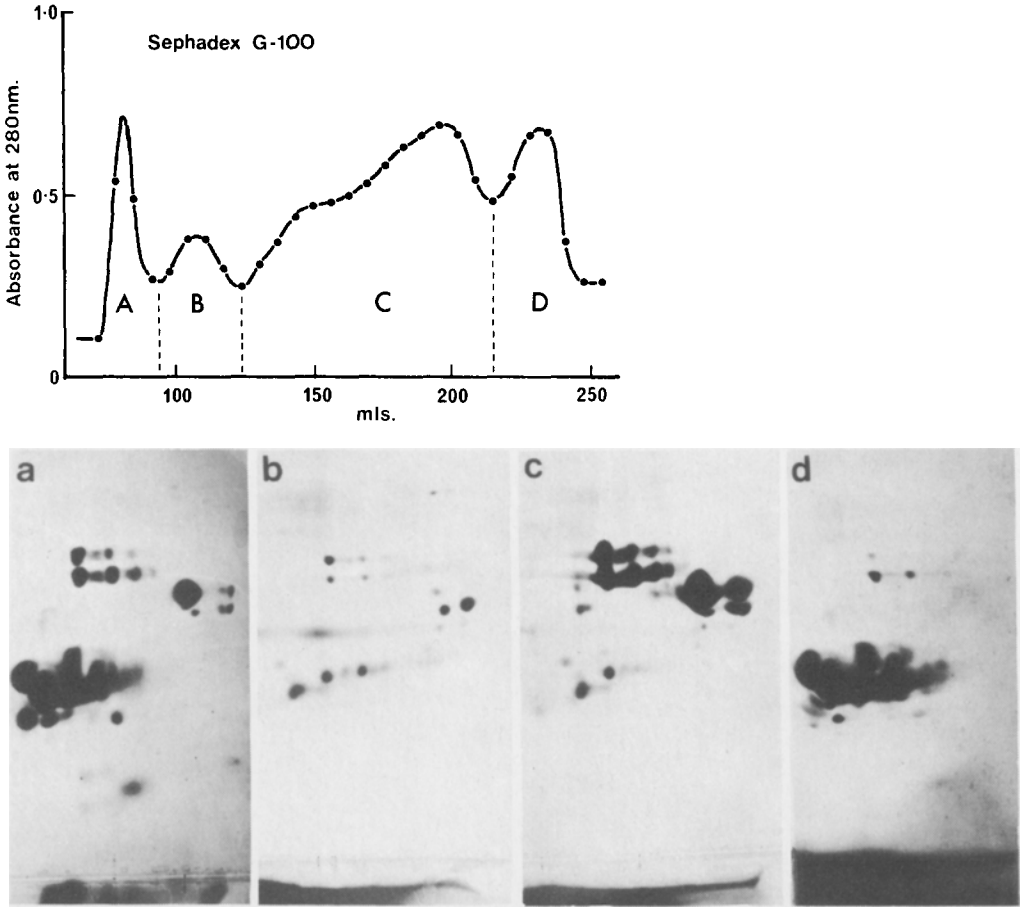

Fig. 3a-d. Above: OD_{280} scan of Sephadex G-100 gel filtration fractionation of a 70% ethanol extraction of 'Chinese Spring' endosperm proteins. *A* high molecular weight (> 100,000 daltons) gliadin fraction, *B* high molecular weight gliadin and ω-gliadin fraction (60,000-80,000). *C* α, β, and γ gliadin fraction, and *D* albumin and globulin fraction. Below: Two-dimensional electrophoretic protein subunit pattern of Sephadex G-100 fractions. **a** total 70% ethanol extract; **b** Fraction A; **c** Fraction B; **d** Fraction C. (1200 μg protein per gel)

A comparison of the two-dimensional patterns of N6DT6A (Fig. 7a) or N6DT6B (Fig. 7b) with that of 'Chinese Spring' euploid (Fig. 2) shows the absence of subunits 17 and 18 (Fig 2) with a similar molecular weight of 35,000. Their presence in the pattern of ditelosomic 6Dα (Fig. 7c) and absence from that of ditelosomic 6Dβ (Fig. 7d) suggest that the alpha arms of chromosome 6D control their production. The subunits absent from these various aneuploid lines involving Group 6 chromosomes are summarised in Table 1. No new subunits were observed in the protein patterns of these aneuploid lines.

The Control of Grain Proteins by Group 2 Chromosomes – Aneuploid Lines

The subunit electrophoretic patterns of proteins extracted from nullisomic-tetrasomic stocks involving homoeologous chromosomes of groups 2-5 and 7 have been studied, but only some of the aneuploids involving Group 2 chromosomes and two lines involving Group 4 chromosomes showed marked differences. The two-dimensional subunit pattern of N2BT2A endosperm was very different from 'Chinese Spring' in the seed stock maintained at Cambridge and very different from a sample of N2BT2A recently obtained from Dr. J. Beitz. Interpretation of these aneuploid stocks must therefore await further clarification of their genotypes. N2DT2A (fig. 8b) and tetra-2A (Fig. 8c) have some protein subunits absent in comparison with the euploid pattern. The subunits absent in N2DT2A (Fig. 8b) and tetra-2A (Fig. 8c) are those also absent from N6DT6A, N6DT6B and ditelosomic 6Dβ (Fig. 7a, b and d). N2BT2D, N2DT2B, tetra-2B and tetra-2D show no protein subunit differences from 'Chinese Spring' (gels not shown). These results are summarised in Table 1. No subunits have been observed in these aneuploid stocks which are not visible in the euploid.

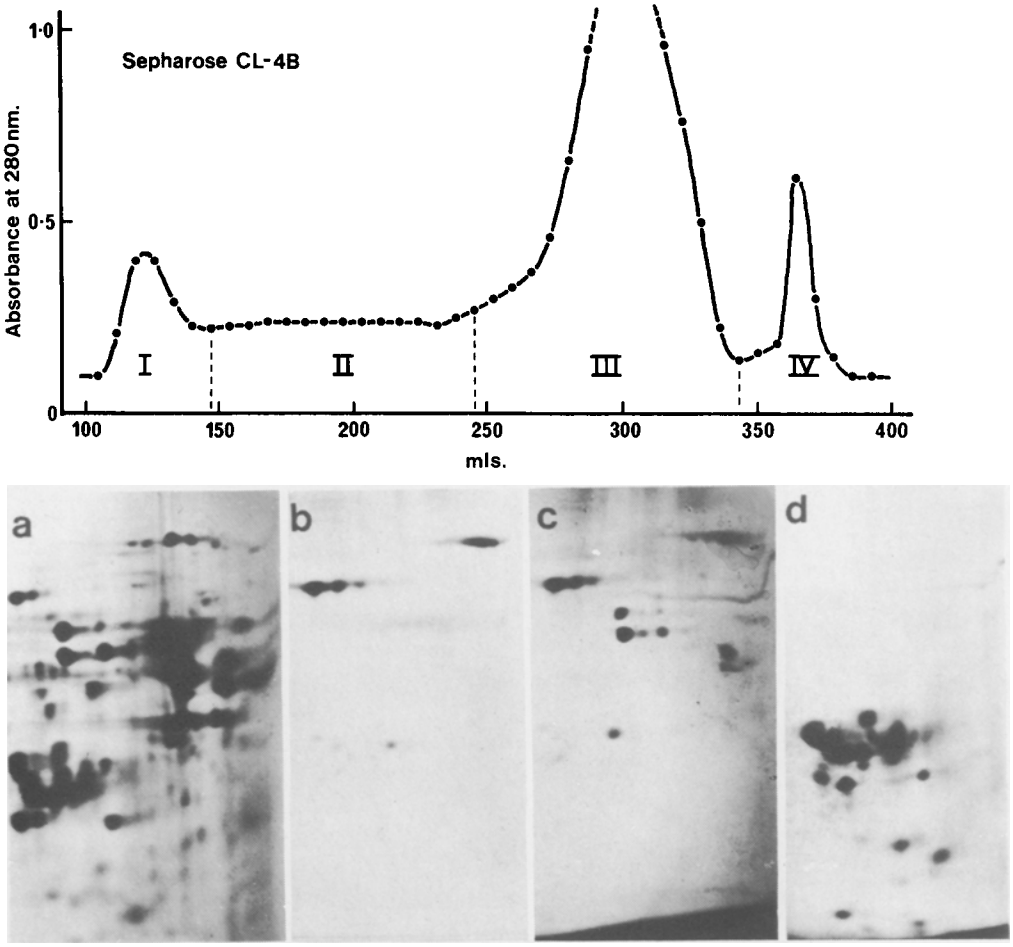

Fig. 4a-d. Above: OD_{280} scan of Sepharose CL-4B gel filtration fractionation of total endosperm protein. I – glutenin fraction, II – glutenin and gliadin fraction, III – gliadin, albumin, and globulin fraction, and IV – albumin and globulin fraction. Below: Two-dimensional electrophoretic protein subunit patterns of Sepharose CL-4B fractions. a total extract; b Fraction I; c Fraction II; d Fraction III. (1200 μg protein loaded per gel)

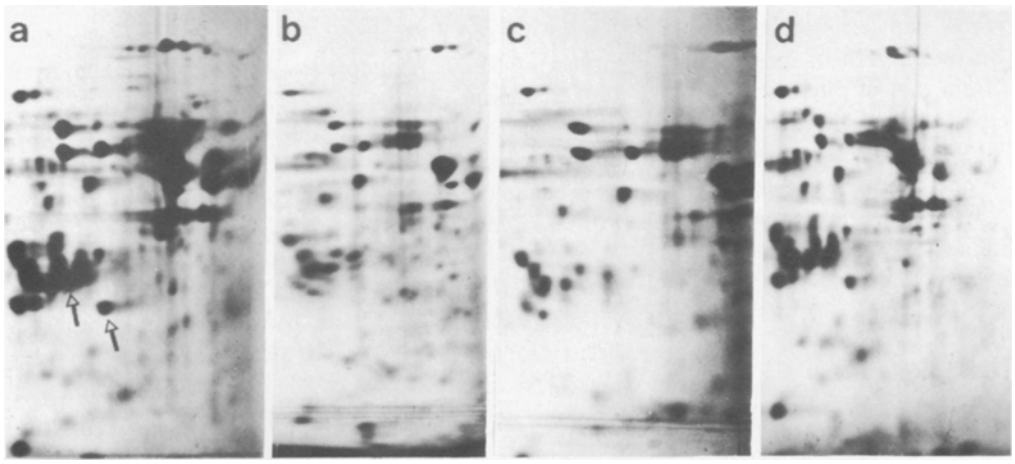

Fig. 5a-d. Two-dimensional electrophoretic protein subunit patterns of aneuploids of 'Chinese Spring'. a CS euploid; b N6AT6B; c N6AT6D; and d DT6A^S Arrows show the position of protein subunits deleted in N6AT6B and N6AT6D. (1200 μg protein per gel)

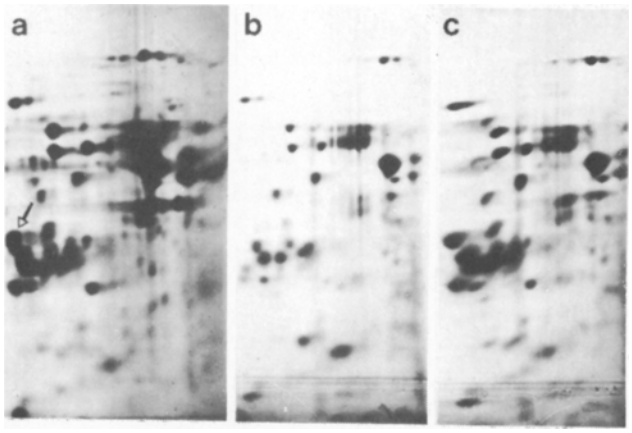

Fig. 6a-c. Two-dimensional electrophoretic protein subunit patterns of aneuploids of 'Chinese Spring'. **a** CS euploid; **b** N6BT6A; and **c** DT6B^{S}. The arrow shows the position of the protein subunit deleted in N6BT6A. (1200 μg protein per gel)

The Control of Grain Proteins by Group 2 Chromosomes – Chromosome Substitution Lines

Of the 33 intervarietal and interspecific chromosome substitution lines studied (see Materials and Methods), only two: CS ('Hope' 2D) and CS ('Timstein' 2D) (Fig. 9b, c) differed in their subunit patterns from 'Chinese Spring' euploid (Fig. 9a). The patterns of CS ('Hope' 2D) and CS ('Timstein' 2D) are very similar to each other, both lacking subunits 17 and 18 (Fig. 2).

The Control of Grain Proteins by Chromosome 4D

Bietz et al. (1975) reported chromosome 4D to control a major protein band of molecular weight 68,000. A protein streak of similar molecular weight is absent from

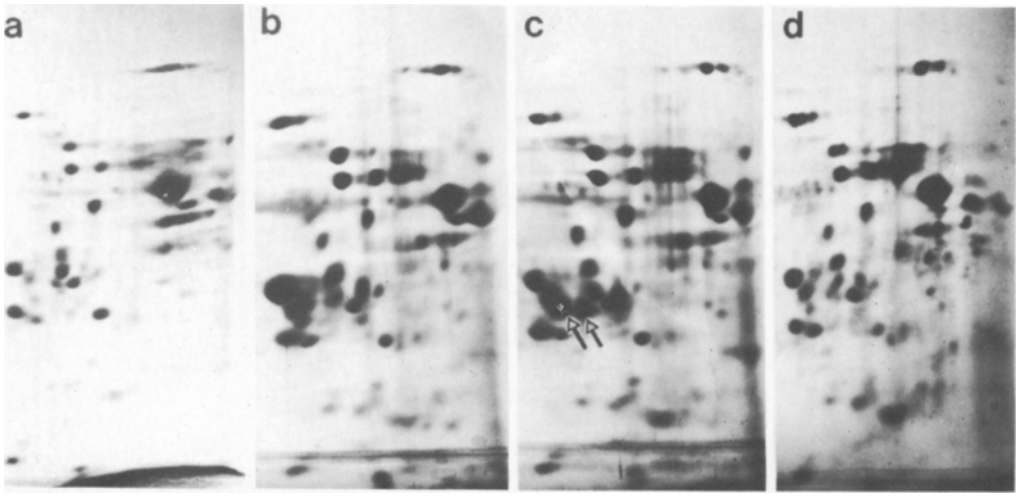

Fig. 7a-d. Two-dimensional electrophoretic protein subunit patterns of aneuploids of 'Chinese Spring'. **a** N6DT6A; **b** N6DT6B; **c** DT6Dα; and **d** DT6Dβ. Arrows show the position of protein subunits deleted in the other lines. (1200 μg protein per gel)

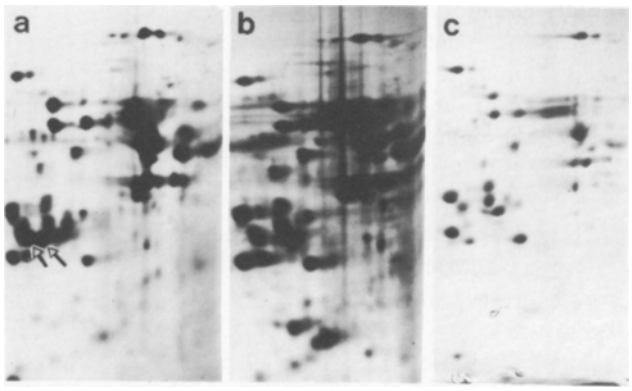

Fig. 8a-c. Two-dimensional electrophoretic protein subunit patterns of anueploids of 'Chinese Spring'. **a** CS euploid; **b** N2DT2A; and **c** T2A. Arrows show the position of deleted protein subunits in the other lines. (1200 μg protein per gel)

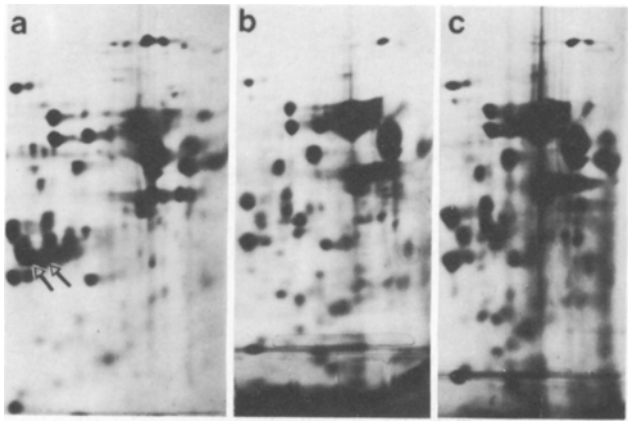

Fig. 9a-c. Two-dimensional electrophoretic protein subunit patterns of aneuploids of 'Chinese Spring'. **a** CS euploid; **b** CS ('Hope' 2D); and **c** CS ('Timstein' 2D). Arrows show the position of deleted protein subunits in the other lines. (1200 μg protein per gel)

Table 1. Summary of protein subunit patterns of some aneuploid stocks of 'Chinese Spring'

	Chromosome dosage						
Genotype	6A	6B	6D	2A	2B	2D	subunits missing (Fig. 2)
CS euploid	2	2	2	2	2	2	none – all present
N6AT6B	0	4	2	2	2	2	20, 24
N6AT6D	0	2	4	2	2	2	20, 24
$6A^s$	short arms	2	2	2	2	2	none
N6BT6A	4	0	2	2	2	2	14
$6B^s$	2	short arms	2	2	2	2	none
N6DT6A	4	2	0	2	2	2	17, 18
N6DT6B	2	4	0	2	2	2	17, 18
DT6Dβ	2	2	alpha arms	2	2	2	none – all present
DT6Dβ	2	2	beta arms	2	2	2	17, 18
N2BT2A	2	2	2	4	0	2	a
N2BT2D	2	2	2	2	0	4	none – all present
N2DT2A	2	2	2	4	2	0	17, 18
N2DT2B	2	2	2	2	4	0	none – all present
T2A	2	2	2	4	2	2	17, 18
T2B	2	2	2	2	4	2	none – all present
T2D	2	2	2	2	2	4	none – all present

[a] Different stocks have yielded different results

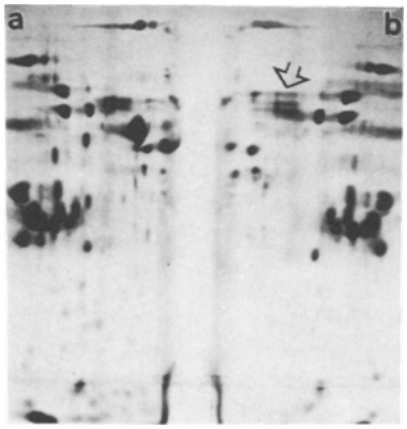

Fig. 10 a and b. Two-dimensional electrophoretic protein subunit patterns of aneuploids of 'Chinese Spring'. **a** N4DT4A and **b** $DT1D^L$. The absence of the 68,000 molecular weight streak of protein in N4DT4A is clearly seen in this mirror image comparison on a single gel. The arrow shows this protein. (1200 μg protein loaded per gel)

the two-dimensional subunit pattern of N4DT4A (Fig. 10a) and N4DT4B (not shown) thus confirming the control of this protein by chromosome 4D. The protein streak is present in the NaCl extract of the modified Osborne extraction (Fig. 1c) and therefore appears to be a high molecular weight gloublin.

Discussion

Classification of Storage Protein Subunits of the Two-Dimensional Electrophoretic Pattern of 'Chinese Spring'

A number of the protein subunits of 'Chinese Spring' are found in more than one fraction in each of the fractionation procedures. The 'overlap' of certain subunits from the gliadin and glutenin fractions in the Osborne fractionation illustrates the confusion which was built up around the terms 'gliadin' and 'glutenin'. Nevertheless, important conclusions can still be drawn.

Subunits 1 and 2 (Fig. 2) can clearly be classed as glutenin subunits, as they are present only in the acetic acid extract of the modified Osborne extraction and fraction 1 of the Sepharose CL-4B fractionation. They are not present in the 70% ethanol 'gliadin' extractions. Subunits 3-9 and 12 (Fig. 2) appear to be gliadin subunits and have molecular weights between 60,000 and 80,000 typical of ω-gliadins (Bietz and Wall 1972; Booth and Ewart 1969; Charbonnier 1973). Subunits 13-24 (Fig. 2) are α, β and γ gliadins.

Traces of subunits 13, 14, 16-19, and 22 (Fig. 2) were found in the glutenin I and II fractions of the Sepharose CL-4B separation (Fig. 4). Small amounts of low molecular weight gliadin subunits in the Group numbered 13 to 23 (Fig. 2) were also found in the high molecular weight

gliadin fraction. The presence of similar low molecular weight subunits in these two high molecular weight fractions has been previously reported (Bietz and Wall 1972, 1973; Payne and Corfield 1979). These low molecular weight subunits therefore appear capable of strong in vivo associations between themselves and higher molecular weight subunits, apparently by disulphide bonds which are reduced by β-mercaptoethanol.

Control of Protein Subunits by Group 1 and 6 Homoeologous Chromosomes

The electrophoretic system described by Brown et al. (1979) has now been used to show that chromosomes 6A, 6B and 6D control at least five major grain protein subunits (see Table 1). This is a minimum number because (1) some subunits, especially those numbered 13-24 (Fig. 2) are not particularly well separated and each protein spot resolved may consist of several subunits, (2) some subunits may have isoelectric points outside the range we have selected for study, and (3) if different subunits with identical electrophoretic behaviour in the system are specified by genes on different chromosomes (e.g. homoeologous), they could not be assigned to chromosomes. Wrigley and Shepherd (1973) allocated the genetic control of 20 of their 46 gliadins to homoeologous Group 6 chromosomes. The differences between their extraction and electrophoretic systems and those used here have been discussed previously (Brown et al. 1979). It seems reasonable to assume that the structural genes for these five major subunits lie on Group 6 chromosomes since the subunits consistently disappeared on removal of the particular Group 6 chromosome, yet are not consistently deleted when any of the other 20 chromosomes are deleted.

In our previously reported study (Brown et al. 1979) the presumed structural genes for 14 subunits or groups of subunits have been assigned to chromosome 1A (subunit no. 10), 1B (subunit nos. 3-5, 12 and one component of 6), and 1D (subunit nos. 1, 2, 7-9, 11 and two components of 6) (Fig. 2). Thus 19 major subunits of those numbered in Figure 2 are specified by chromosomes of Groups 1 and 6 of 'Chinese Spring' wheat (see Table 1). The remaining 12 subunits (13, 15, 16, 19, 21-23, and 25-29) are presumably specified by genes duplicated on homoeologous chromosomes.

From the classification of subunits established in this paper the following general conclusions appear valid: at least some of the α, β and γ gliadins (molecular weights SDS-PAGE 30,000 to 40,000) are specified by the short arms of Group 6 chromosomes, the ω gliadins (molecular weights SDS-PAGE 50,000 to 70,000) are specified by the short arms of Group 1 chromosomes while the glutenin subunits (molecular weights SDS-PAGE $>$ 85,000) are specified by the long arms of Group 1 chromosomes. These conclusions support the idea that the members of the different groups of gliadin and glutenin subunits evolved by gene duplication into clustered arrays.

The finding in this paper that the 68,000 molecular weight protein controlled by chromosome 4D (Bietz et al. 1975) appears to be a globulin protein substantiates the hypothesis that all gliadin and glutenin subunits are controlled by Group 1 and 6 chromosomes.

Regulation of Grain Protein Subunit Levels by Group 2 Chromosomes

The results in Table 1 also include the aberrant subunit patterns found in two of the aneuploids involving homoeologous Group 2 chromosomes. Because it is believed that the structural genes for the subunits in question lie on chromosome 6D, and assuming that the genotypes of the Group 2 aneuploids are what they are supposed to be, the Group 2 effects would appear to be of a regulatory nature, dependent on chromosome dosage. There seems no obvious reason why in the production and/or maintenance of the two aneuploid lines in question tetra-2A and N2DT2A, the structural genes on chromosomes 6D should have been lost but this must be verified.

The Group 2 chromosome results relate to and extend those of Shepherd (1968) who examined N2DT2A and tetra-2A stocks by one-dimensional electrophoretic analysis. He found one gliadin band deleted in these lines and also in N6DT6A and N6DT6B. This band could correspond to subunits 17 and 18 (Fig. 2). The production of the new band observed by Shepherd (1968) was not seen here.

The two Group 2 aneuploid lines which illustrate variation in the subunit composition of the 'Chinese Spring' storage proteins all contain four doses of chromosome 2A. This may be the basis of a regulatory system assuming that chromosome 2A is acting similarly in the two lines. The genotypic difference between N2DT2A and tetra-2A is the absence of chromosome 2D in the former stock. Because the protein subunit patterns of the two stocks do not differ, chromosome 2D is probably not involved in any regulatory system. Thus, four doses of chromosome 2A may be the source of the inhibition of the 6D α-controlled subunits 17 and 18.

The intervarietal and interspecific chromosome substitution lines were investigated to see if any further evidence could be gained for a regulatory system involving Group 2 chromosomes. The chromosome 2B and 2D substitution lines were studied especially, because if the new chromosme carried allele(s) similar to that on chromosome 2A responsible for the aberrant subunit patterns,

then the genetic complement would be equivalent to tetrasomic 2A and loss of specific subunit groups might be observed. Of the thirty-two Group 2 substitution lines examined, only two, CS ('Hope' 2D) and CS ('Timstein' 2D) showed close similarities to tetrasomic-2A and N2DT2A. Thus it is possible that chromosomes 2D of the varieties 'Hope' and 'Timstein' have alleles of regulatory effect similar to those on chromosome 2A of 'Chinese Spring'. Nevertheless, proof of whether there is a major protein-specific regulatory system involving the dosage of Group 2 chromosomes requires evidence that the genes for subunits 17 and 18 (Fig. 2) are present in the relevant stocks where subunits 17 and 18 could not be detected. This is currently being investigated.

In summary then with regard to the regulation of the major endosperm proteins, the dominant conclusion is that alteration of the dosage of any one of the chromosomes of 'Chinese Spring', with the exception of chromosome 2A or those chromosomes carrying the structural genes, does not lead to loss of, or to a major change in, the properties of any of the subunits easily discerned by two-dimensional electrophoresis. It is also pertinent to note that only two of the thirty-three Group 2 substitution lines resulted in any discernible changes in subunit composition while substitution of Group 1 and Group 6 chromosomes produces a wide spectrum of subunit variation (Brown et al. 1979, 1981). Thus it still seems reasonable to conclude that the chromosomes of Groups 1 and 6 are responsible for the major controls of storage protein structure and amounts in wheat but clearly information on other chromosomes may play minor roles.

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