Two-Dimensional Fractionation of the Endosperm Proteins of Bread Wheat (*Triticum aestivum*): Biochemical and Genetic Studies

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ABSTRACT

Wheat endosperm protein, extracted in the presence of 2-mercaptoethanol and sodium dodecyl sulfate, fractionated into about 100 components of molecular weight greater than 25,000 by two-dimensional polyacrylamide gel electrophoresis under dissociating conditions. These components were grouped into high molecular weight glutenin subunits, low molecular weight glutenin subunits (two groups), gliadins, high molecular weight albumins, globulins, nonstorage proteins, and triplet-band subunits (two groups). The triplet-band proteins were very recently discovered by electrophoresis under nonreducing conditions. The chromosome and chromosome-arm location of most components was determined using genetic lines of Chinese Spring. A minor, basic protein of molecular weight similar to gliadins and low molecular weight glutenin subunits was shown to be controlled by genes on the long (L) arm, rather than the short arm, of chromosome 1A. It only occurred in those varieties which lacked a 1AL-encoded high molecular weight subunit of glutenin. The possible effects on breadmaking quality of non-prolamin type storage proteins are discussed.

The starchy endosperm of mature wheat grains contains several types of protein including the storage proteins gliadin and glutenin, proteins and enzymes that have survived from the metabolically active endosperm of the developing grain, and structural proteins, such as those in membranes.

Although there have been many electrophoretic studies of the endosperm proteins, very few have clearly distinguished all the above protein groups. Currently, the most satisfactory way of analyzing total protein is by using a combination of the two, two-dimensional fractionation procedures of O'Farrell (O'Farrell 1975, O'Farrell et al 1977). The first procedure, which uses isoelectric focusing (IEF) in the first dimension and sodium dodecyl sulfate, polyacrylamide gel electrophoresis in the second (IEF × SDS-PAGE), was first exploited by Brown and co-workers to study wheat endosperm proteins (Brown et al 1979, Brown and Flavell 1981, Brown et al 1981). The procedure was later modified by Holt et al (1981) to give slightly improved resolution, and was combined with the second procedure of O'Farrell et al (1977) in which nonequilibrium pH gradient electrophoresis (NEPHGE) replaces IEF in the first dimension to additionally resolve those proteins with isoelectric points greater than 7.5. Approximately 60 major and 100 minor components were detected. In a later study (Payne et al 1984), the fractionated proteins were subgrouped into various areas of the two-dimensional map on the basis of various biochemical properties. The groups were high molecular weight (HMW) glutenin subunits, low molecular weight (LMW) glutenin subunits (two groups), gliadins, globulins, and nonstorage proteins.

In this paper, the two-dimensional protein map obtained by the NEPHGE × SDS-PAGE and IEF × SDS-PAGE two-dimensional procedures has been subdivided further according to the biochemical and genetic properties of the proteins. The genes controlling several nonstorage proteins were assigned to chromosomes for the first time.

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MATERIALS AND METHODS

The genetic lines used in this study were obtained from and maintained by the Cytogenetics Department of the Plant Breeding Institute. Many of the compensating nullisomic-tetrasomic lines of Chinese Spring wheat developed by Sears (1954) were analyzed. In line N1A T1D for example, the pair of 1A chromosomes (nulli) are replaced by a homoeologous pair (tetra), in this case chromosome 1D. To study the chromosome arm location of endosperm protein genes, ditelosomic lines were examined. A ditelosomic line has the chromosome arms of a pair of chromosomes missing, the short arms of chromosome 1A in the case of Chinese Spring, ditelosomic 1A long (CS DT 1AL). Finally, several intervarietal chromosome substitution lines were examined, such as Chinese Spring (Hope 1A). This line contains the chromosomes of euploid Chinese Spring except for the pair 1A chromosomes, which are replaced by homologous chromosomes from variety Hope.

Two-Dimensional Electrophoresis

The two-dimensional procedure involved two separate first dimensions and a common second dimension. Most proteins could be fractionated in the procedure using IEF in the first dimension and SDS-PAGE in the second. The more basic proteins were separated using NEPHGE in place of IEF. The methods, originally described by O'Farrell (1975) and O'Farrell et al (1977), have been modified further for the separation of wheat proteins (Jackson et al 1983).

Preparation of Albumins and Globulins

Samples for extraction weighed approximately 50 mg and consisted of one or two grains with their embryos discarded. Each

sample was crushed with smooth-jawed pliers and placed in a 1.5-ml disposable centrifuge tube. One milliliter of 0.5*M* sodium chloride, 0.05*M* sodium phosphate buffer, pH 7.2, was added and the samples mixed gently on a rotator (2 rev/min) for 2 hr at 4°C. The samples were centrifuged for 5 min using an Eppendorf centrifuge and the supernatants transferred to fresh tubes. The lids were removed and the open mouths of the tubes were covered with a sheet of dialysis tubing and fixed with rubber bands. The tubes were dialyzed against a large volume of distilled water at 4°C for 24 hr. The samples were centrifuged for 5 min and the supernatants (the albumin fractions) removed and freeze dried. The pelleted globulins were surface rinsed with water to reduce albumin contamination and similarly freeze dried.

RESULTS

Protein Groups in the Endosperm

Figure 1 summarizes our attempt at classifying the endosperm proteins of the mature endosperm of wheat. The predominant proteins are the storage proteins, described here as prolamins as advocated by Shewry et al (1984) because of their high content of *pro*line and glut*amine* (Kasarda et al 1976, for review). In the developing endosperm they are deposited in membrane-bound protein bodies (Field et al 1983). These proteins are subdivided into gliadin and glutenin. The gliadin fraction is a complex mixture of simple polypeptides. They do not participate in intermolecular disulfide bond formation, and they are not excluded from gel-filtration media designed to sieve molecules of molecular weight lower than 100,000, such as Sephadex G-100 (for example, Payne and Corfield 1979). By lactate-PAGE they typically fractionate into α-, β-, γ-, and ω-gliadins (Woychick et al 1961). N-terminal amino-acid sequencing (Bietz et al 1977, Kasarda et al 1983) and DNA sequencing of gliadin gene sequences cloned in bacteria (Bartels and Thompson 1983, Kasarda et al 1984) have revealed three distinctive groups, two corresponding to ω- and γ-gliadins and the third corresponding to α- and β-gliadins combined—hence the three subdivisions of gliadin in Figure 1.

Glutenin is radically different from gliadin in occurring as large disulfide-linked molecules or aggregates. It elutes with the void volume upon gel-filtration chromatography using Sephadex G-100 (for example, Payne and Corfield 1979), and is only adequately sieved by media with large particles, such as Sepharose (Huebner and Wall 1976). Upon reduction, the aggregates dissociate into approximately 15 subunits that group into HMW and LMW subunits by one-dimensional electrophoresis. By two-dimensional electrophoresis, the LMW subunits subdivide into B, C, and D subunits (Jackson et al 1983). The major group, the B subunits, are basic proteins, whereas the D subunits are minor, have slightly greater molecular weights, and are the most acidic group of proteins in the endosperm. The minor C subunits are a diffuse group of widely different isoelectric points. Evolutionary relationships between them are not known.

In addition to the major storage proteins, there are a myriad of additional, minor proteins, which in total make a significant contribution to total endosperm protein. Many will have, or have had in the developing endosperm, metabolic or structural (membrane) functions. In this paper these proteins having molecular weights less than 25,000 have not been studied. They include the LMW gliadins and the CM proteins of Rodriguez-Loperena et al (1975) and Salcedo et al (1979) and various enzyme-inhibitor proteins (see Kasarda et al 1976). The HMW albumins shown in Figure 1 are defined as being freely soluble in neutral, distilled water, and the globulins are soluble in neutral, 0.5–1.0*M* sodium chloride at 4°C but insoluble in distilled water. The function of these two groups of proteins has not been studied. The remaining group, the nonstorage proteins (Fig. 1), have been defined mainly in terms of their electrophoretic and solubility properties, and these will be described later.

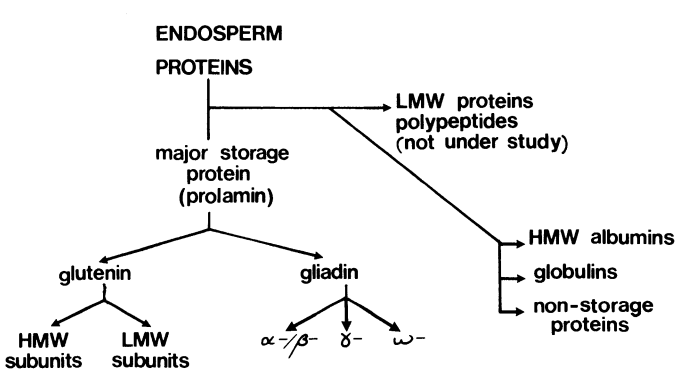

Fig. 1. Classification of the major endosperm proteins of wheat.

Characterization by Two-Dimensional Electrophoresis

A two-dimensional fractionation of the total endosperm proteins of variety Chinese Spring is shown in Figure 2. The two-dimensional protein map has been divided into areas according to the biochemical and genetic properties of the proteins and is similar, although more detailed, than that published previously (Payne et al 1984). The subdivision of the map into gliadin, HMW glutenin subunits, LMW(B), and LMW(D) subunits is taken from the work of Jackson et al (1983) which was primarily based on the electrophoretic analysis of Sephadex G-100 fractions.

A two-dimensional electrophoretic separation of the albumin protein fraction by IEF × SDS-PAGE is shown in Figure 3A. Numerous protein components are present, many of low molecular weight and not seen clearly in total protein extracts (Fig. 2). The major proteins in this fraction occur as three characteristic streaks, and these are labeled HMW albumins in Figure 2. They were also seen clearly in separations of albumins by one-dimensional SDS-PAGE (Cole et al 1981). The proteins are not, therefore, globulins as stated in error by Payne et al (1984). As might be expected, all glutenin subunits and most gliadins are absent in Figure 3A, but the ω-gliadin coded by chromosome 1D and four other gliadins are present in relatively small amounts and are arrowed. Not shown here is the fractionation by NEPHGE × SDS-PAGE, as only one additional, major albumin of high molecular weight was present.

Another group found to be present in significant amounts is the "nonstorage proteins" of Payne et al (1984); these have been boxed in, in Figure 3A. Preliminary studies on their solubility properties are rather puzzling. Although the nonstorage proteins are found in water-soluble extracts, they do not extract quantitatively. In successive extractions with dilute salt, 70% ethanol, and 0.1*N* acetic acid a significant amount of this group remains in the final, insoluble residue. They are particularly insoluble in urea solutions, and subcellular fractionation in media used to isolate polyribosomes indicates that a portion of them are associated with subcellular particles that sediment at about 27,000 × g_{max} for 30 min, whereas the rest occur in the postribosomal supernatant. The HMW albumin streaks are much more soluble in water, and in subcellular fractions they occur mainly in the postribosomal supernatant. For these reasons the nonstorage proteins and the HMW albumins will be regarded as separate groups, at least until they have been characterized further.

The true globulin proteins of the endosperm have near-neutral isoelectric points, so they separate almost entirely on both of the IEF and NEPHGE first dimensions (Figs. 2 and 3B). Of the two procedures, NEPHGE resolves all of the proteins except the one which is the most acidic; a separation of NEPHGE × SDS-PAGE is shown in Figure 3B. Because the globulins are present in such small amounts in the endosperm they are rarely seen in fractionations of total protein extracts, but their general location on the two-dimensional map is shown in Figure 2. There are about 24 major globulin components (Fig. 3B), several of which occur in subgroups with the same mobility in the second dimension. It is not known whether they arise as artifacts during the experimental procedure or whether they are distinctive proteins coded by different genes.

Another protein group in Figure 2 that has been identified since the previous publication of Payne et al (1984) is the so-called "triplet band" region described by Singh and Shepherd (1984). These authors showed that endosperm proteins, fractionated by

SDS-PAGE in the absence of a reducing agent, contained three minor, closely associated bands of slow mobility. The authors showed that the protein in each band is built up from two large and two small subunits that are disulfide linked, and it is these subunits which are labeled in the two-dimensional map of Figure 2.

The triplet-band proteins have not been fully characterized biochemically, and they cannot yet be included in the classification scheme shown in Figure 1. It is not known whether they are true storage proteins like glutenin and gliadin, and are present in protein bodies in the developing endosperm, or whether they occur elsewhere and have some other function. If they are storage proteins then they do not fall clearly into the gliadin or glutenin groups. Unlike gliadin, they are insoluble in 70% aqueous ethanol at room temperature and they have a disulfide-bonded subunit

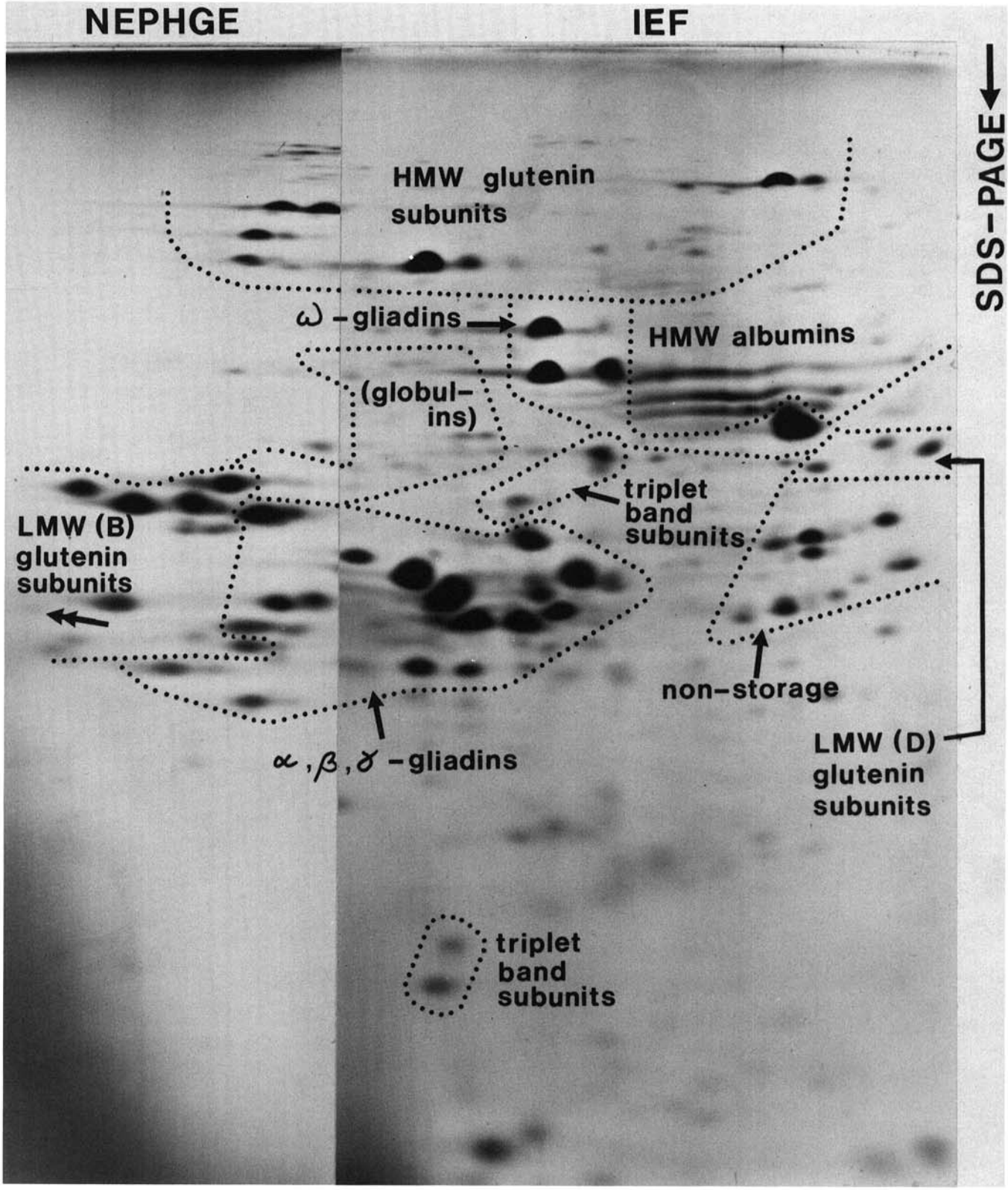

Fig. 2. Fractionation of endosperm proteins by nonequilibrium pH gradient electrophoresis × sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing × SDS-PAGE. The two first dimensions have a partially overlapping pH gradient so that a few proteins occur on both systems. The overlapping areas of the gel photographs have been removed to give a continuous pH gradient.

structure. Unlike glutenin, the subunits of the triplet bands are only disulfide linked into very small aggregates.

Chromosomal Location of Controlling Genes

In order to locate endosperm protein genes to chromosomes, many compensated nullisomic-tetrasomic lines of Chinese Spring and ditelosomic lines were analyzed by NEPHGE × SDS-PAGE and IEF × SDS-PAGE. As expected from the work of several groups (Payne et al 1982, for review) the genes for HMW and LMW glutenin subunits were located on the long arm and short arms, respectively, of the group 1 chromosomes (Fig. 4). In addition, the gliadin genes were located on the short arms of the group 1 and on the group 6 chromosomes (see Fig. 4). However, an unexpected finding was that a minor, basic protein of similar mobility by SDS-PAGE to LMW glutenin subunits was coded by genes on the long instead of the short arm of chromosome 1A (Fig. 2; double-headed arrow, Fig. 4). None of the HMW albumins, the globulins, or the nonstorage proteins were deleted by any of the group 1 or group 6 nullisomic-tetrasomic lines, indicating that their controlling genes are probably not located on chromosomes 1A, 1B, 1D, 6A, 6B, or 6D. However, both the large and small subunits of the triplet-band proteins are controlled by genes on the short arms of chromosomes 1A and 1D (Fig. 4) as shown by Singh and Shepherd (1984).

In agreement with Brown and Flavell (1981) the HMW albumin streak with slowest mobility in the second dimension (Fig. 2) is controlled by genes on chromosomes 4D. However, these authors misclassified the protein products as globulins. Our work with ditelosomic lines shows that the genes are located on the long arm of this chromosome (Fig. 4). The protein probably corresponds to the 4DL-encoded protein described by Bietz et al (1975) as an HMW glutenin subunit. It is known that these proteins, although inherently water soluble, denature easily and can then become associated with glutenin fractions prepared by differential solubility (Payne and Corfield, *unpublished data*). The genes for the two remaining HMW albumin proteins could not be located to chromosomes, perhaps because their coding sequences are more conserved than the storage proteins so that proteins arising from different genomes have identical two-dimensional map positions.

The genes for the nonstorage proteins were located to chromosomes for the first time (Fig. 4). They occur mainly on chromosomes 5AL, 5B, and 5DL and might therefore represent a homologous series on the long arms of these chromosomes. There is additionally a major component coded by chromosome 7DS.

Most of the globulin proteins are controlled by genes on the homologous group 4 and group 5 chromosomes. There appears to be a homologous series of globulin genes on 5AL, 5BL, and 5DL. Several other genes have definitely been assigned to chromosomes

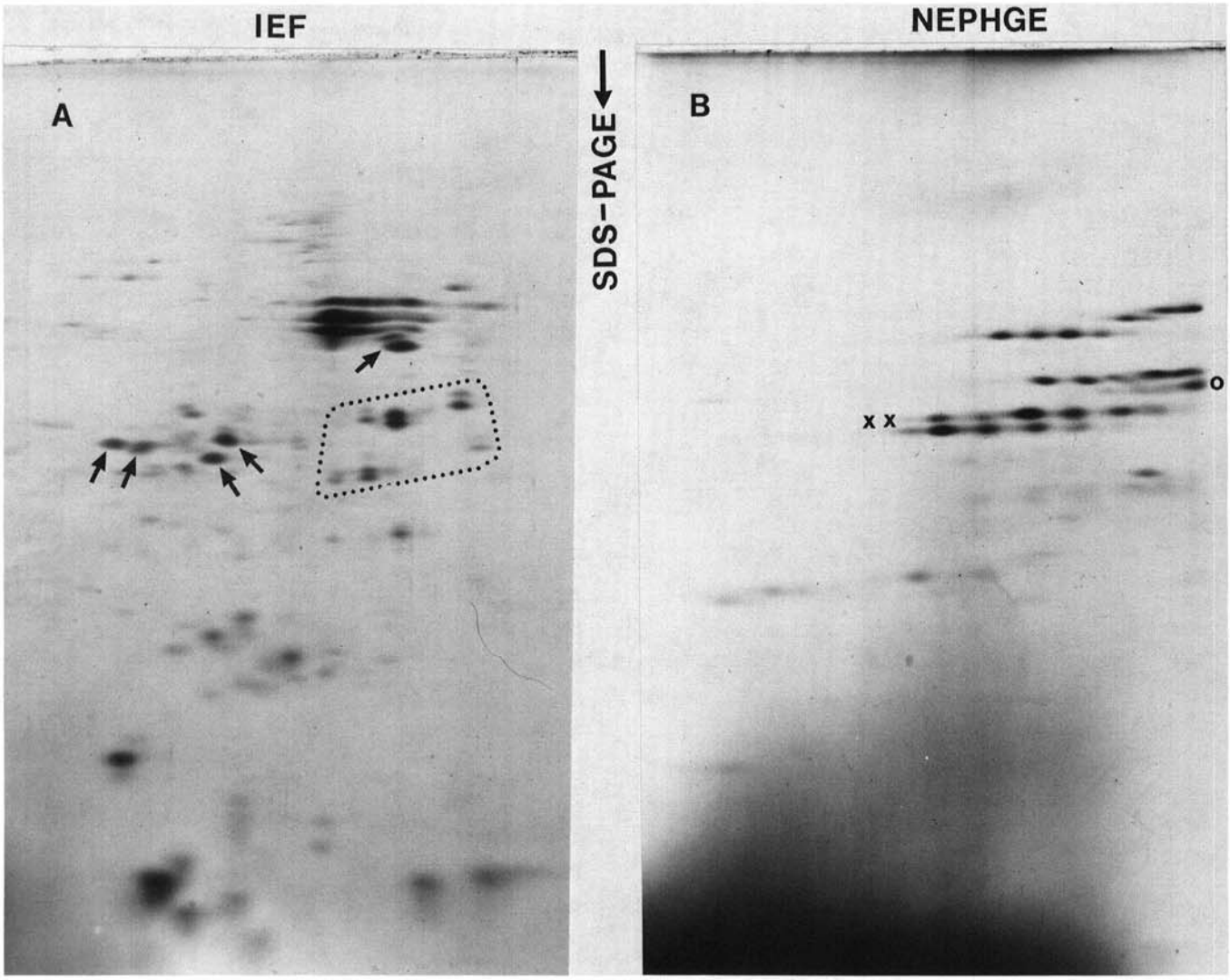

Fig. 3. Fractionation of **A** albumin proteins by isoelectric focusing × sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and **B** globulin proteins by nonequilibrium pH gradient electrophoresis (NEPHGE) × SDS-PAGE. The contaminating gliadins in **A** are arrowed and the nonstorage proteins are boxed in. Unlike **A**, which is Chinese Spring (CS) euploid, **B** is a globulin extract from CS N4D T4A, so that two components coded by genes on chromosome 4D are missing and are marked by crosses. A chromosome 5D encoded globulin is too acidic to be present on NEPHGE and is marked by an open circle.

4B and 4D. Of the remaining globulins (Fig. 4) at least some are suspected to occur on chromosome 4A. Unfortunately, N4A T4B and N4A T4D are sterile, so grain was not available for testing. However, a nullisomic 4A of the line Dwarf A was shown to lack several globulin components compared to the euploid variety (*unpublished data*). Analysis of group 4 ditelosomics of Chinese Spring showed that the globulin genes occurred on 4BL and 4DS, which, in spite of the terminologies, are homologous chromosome arms (Sears and Sears 1978).

Electrophoretic Analysis of Endosperm Proteins from Different Varieties

Several different varieties and intervarietal chromosome substitution lines were analyzed by NEPHGE × SDS-PAGE and by IEF × SDS-PAGE. For all cases a similar complexity of protein spots was obtained to that shown in Figure 2, but the distribution of spots was often different, showing intervarietal variation for protein type. As reported previously, variation is extensive among gliadins (Zillman and Bushuk 1979) and HMW subunits of glutenin (Payne and Lawrence 1983) and is due to allelic variation in their structural genes. In our work, we have also shown extensive variation among the B group of LMW glutenin subunits (results not shown). However, variation among the HMW albumins, the globulins, triplet bands, and the D group of LMW glutenin subunits is very much less. Thus, the slowest of the albumin streaks (Fig. 3A) coded by genes on chromosome 4DL (Fig. 4) is occasionally deleted, as in Hobbit and Cappelle-Desprez, and in many varieties the two other albumin streaks migrate slightly closer together than they do in Chinese Spring. So far no other variation has been detected for this group.

Other genotypes were also examined for the presence of the protein for which synthesis is controlled by chromosome 1AL and which was provisionally grouped as a LMW glutenin subunit (Figs. 2 and 4). In Figure 5 the proteins of Chinese Spring and the intervarietal chromosome substitution line Chinese Spring (Hope 1A) have been compared after electrophoresis by NEPHGE × SDS-PAGE. The substitution line, which is genetically identical to Chinese Spring except that its 1A chromosome has been replaced by the homologous chromosomes from Hope, does not contain the 1AL-LMW subunit, unlike Chinese Spring. The other difference between the two genotypes that involves the long arm of chromosome 1A is that Chinese Spring (Hope 1A) contains subunit 1, an HMW glutenin subunit coded at *Glu-A1*, whereas Chinese Spring does not produce a recognizable HMW glutenin subunit from genes at this locus (Payne and Lawrence 1983).

In further analyses, Chinese Spring (Timstein 1A) was shown to contain subunit 1 but to lack the 1AL-LMW subunit, similar therefore to the Chinese Spring (Hope 1A) substitution line, but Chinese Spring (Cappelle-Desprez 1A) lacks subunit 1 and contains subunit 1A-LMW. Bezostaya-1 differs from Hope and Timstein in containing subunit 2*, which is allelic to subunit 1 (Payne and Lawrence 1983). The substitution line, Cappelle-Desprez (Bezostaya 1A), like lines containing subunit 1, lacked the 1AL-LMW subunit (not shown). In a limited survey of unrelated varieties (Table I) a strong association was found between the presence of the small, basic subunit controlled by 1AL and the absence of HMW glutenin subunits 1 and 2*. The only apparent exception was Koga II, which lacks both a chromosome 1A-encoded HMW glutenin subunit and 1AL-LMW.

DISCUSSION

Wheat flour is used to make a multitude of different foods depending upon the biochemical and biophysical properties of the flour and the regions in the world where it is being processed. To make leavened bread a strong flour is required which, after mixing with water to form a dough, has elasticity (conferred by glutenin) and extensibility (mainly conferred by gliadin). It is generally accepted that for wheats of low protein content (about 8 or 10%) in the endosperm, the flour is often insufficiently strong to make bread because the glutenin is not optimally elastic. In our work, we have shown that allelic variation at the *Glu-1* loci, which code for

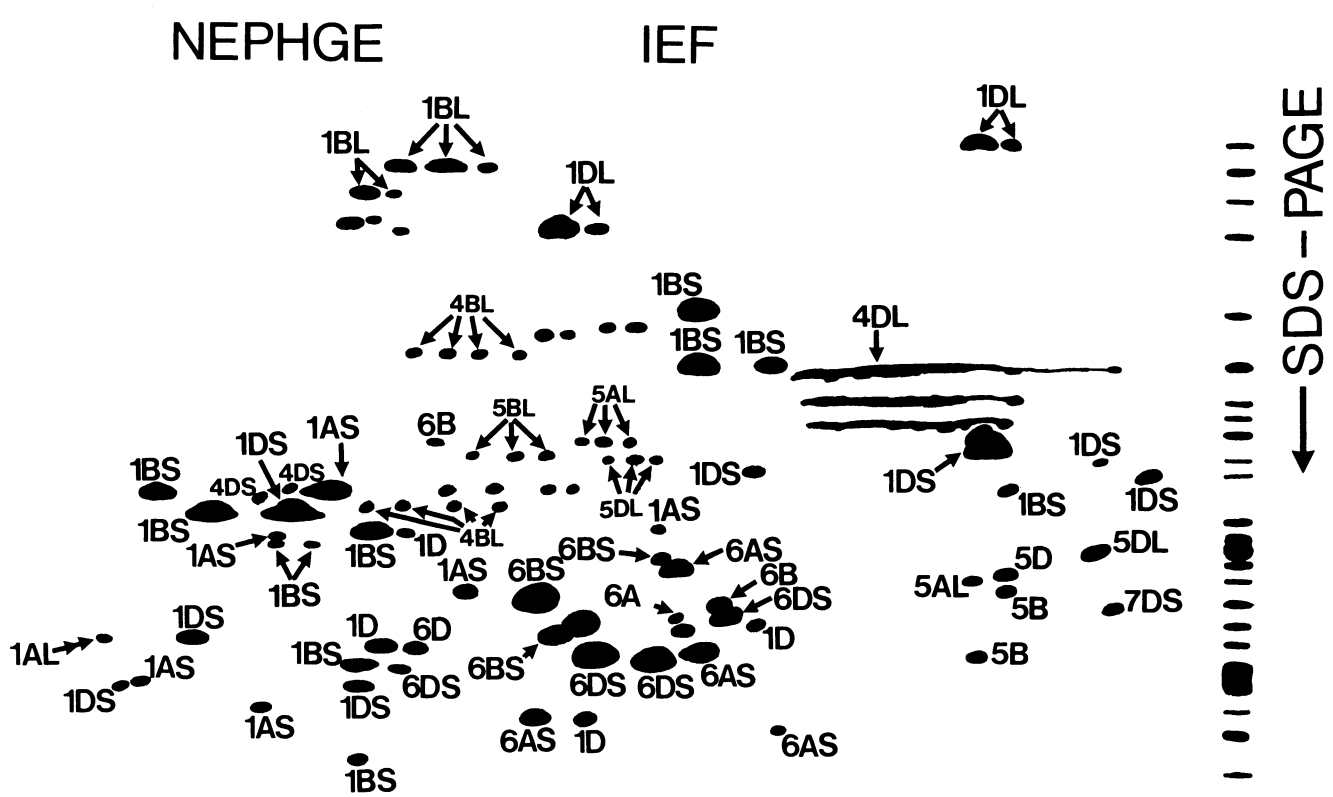

Fig. 4. Diagram of chromosomal location of the genes controlling the major proteins of Chinese Spring resolved by nonequilibrium pH gradient electrophoresis/isoelectric focusing (NEPHGE/IEF) × sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). L is short for long arm and S for short arm. The intensity of the globulin spots has been increased about fivefold, relative to other components, and their chromosome assignments are given in smaller sized print. On the right-hand side the proteins of this variety have been fractionated by one-dimensional SDS-PAGE.

HMW subunits of glutenin, is primarily responsible for differences in elasticities of doughs of different varieties, originally deduced by the SDS Sedimentation Test (Payne et al 1979, 1981) but more recently by more discriminating procedures including test bakes (Payne et al 1984, and *unpublished data*). At the Plant Breeding Institute, Cambridge, this information is being exploited by wheat breeders in selecting parents with complementary, good quality HMW subunits of glutenin, using SDS-PAGE as a secondary screen to select the desired, elite progeny.

In this paper, we describe a preliminary study of the genetic and biochemical properties of HMW protein groups which are clearly different from the prolamin storage proteins, glutenin and gliadin. At least some of these groups, analyzed here by two-dimensional electrophoresis, have been studied in earlier work that used one-dimensional electrophoresis as a method of characterization. Of particular interest to the bread wheat breeder was the finding of a highly aggregating fraction considered at one time to be a part of the glutenin complex. Even after reduction and alkylation followed by gel-filtration in 4*M* urea (Huebner and Wall 1974), 0.1*M* acetic acid (Arakawa et al 1977) or 6*M* guanidine HCl (Huebner and Wall 1974) it still remained in the aggregated state, presumably by noncovalent bonds. The amount of this fraction in a flour was shown to be inversely related to breadmaking quality (Arakawa et al 1977). It had an amino acid composition unlike prolamin but like water-soluble proteins, being much higher in lysine and aspartic acid/asparagine residues and much lower in glutamine and proline residues (Huebner et al 1974, Arakawa et al 1977). Subsequently, Danno et al (1978) showed that this aggregated fraction probably consisted of insoluble albumins denatured during dough mixing or protein extraction. Examination of the SDS-PAGE fractionations of the above authors certainly show the predominance of the HMW albumins, although the presence or absence of the nonstorage proteins, the globulins, and the triplet bands could not be deduced.

In more recent work Graveland et al (1982) described a unique classification of wheat endosperm proteins and demonstrated one fraction called glutelin which was soluble in 1.5% SDS but

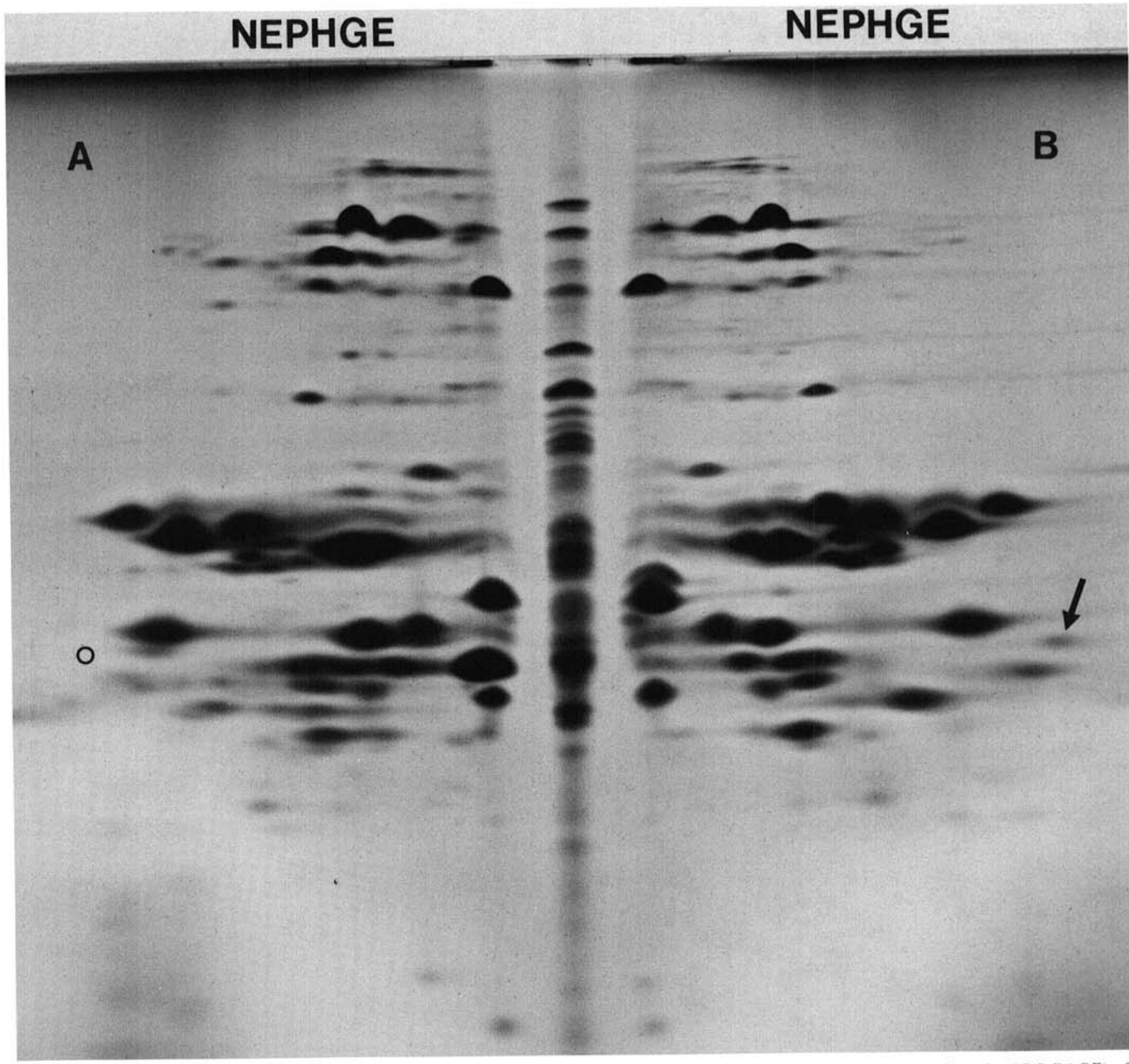

Fig. 5. Fractionation by nonequilibrium pH gradient electrophoresis × sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of **A** Chinese Spring (Hope 1A) and **B** Chinese Spring. The two first-dimensional tube gels were laid head-to-head on the second dimension slab to give two-dimensional patterns that were mirror images of each other. In **B** the 1AL-LMW subunit is arrowed, and in **A** where the subunit is absent, an open circle represents the position it would have taken. The proteins of Chinese Spring have been separated by one-dimensional SDS-PAGE in the center of the gel.

insoluble in water and 5*M* urea. The effect of adding glutelin to a dough was to decrease breadmaking quality. Its amino acid composition was similar to the aggregated fraction above, and analysis by SDS-PAGE showed numerous HMW and LMW components.

It would appear that the HMW albumins are present in the above fractions that impart poor breadmaking properties to doughs, and it is possible that the nonstorage group of proteins is also present, on the basis of its aggregative properties in urea. It is clear that the nonprolamin proteins of the endosperm need to be studied in more detail. Where are they located and what is their function in the developing endosperm?; which are harmful for breadmaking, and what are the relative effects of genotype and growing conditions on their amounts in the mature endosperm? In our study we have shown that the genes coding for many of the nonprolamin groups are not located on the group 1 or group 6 chromosomes and so must be genetically unlinked to the glutenin and gliadin genes. There is a potential, therefore, for wheat breeders to select both for glutenin type and for smaller amounts of deleterious, nonprolamin proteins to achieve further varietal improvement.

The finding that an endosperm protein of similar size to gliadins and LMW glutenin subunits is controlled by genes on the long arm of chromosome 1A rather than the short arms of one of the group 1 or group 6 chromosomes was most unexpected and has not been reported previously. The analyses summarized in Table I show that all varieties containing this protein (called 1AL-LMW) lack an identifiable HMW subunit coded by chromosome 1A, and almost all varieties lacking 1AL-LMW contain one of the two 1A-encoded HMW subunits, 1 and 2*. Chinese Spring is an example of a variety which lacks a 1AL-encoded HMW subunit of glutenin, but recent work involving molecular hybridization of cloned DNA indicates that chromosome 1A nevertheless contains a structural gene (or genes) for a protein of this type (Thompson et al 1983). The HMW subunit genes occur at the locus *Glu-A1* on this chromosome (Payne et al 1984), and the allelic form of the gene in Chinese Spring has been defined *Glu-A1c* (Payne and Lawrence 1983), the so-called null allele. It is a possibility that *Glu-A1c* is not a null allele after all but codes for the small, basic protein 1AL-LMW, presumably as a result of a major alteration in the area of the coding sequence of this gene. Set against this is the result with Koga II (Table I) and the finding that 1AL-LMW only stains faintly with Coomassie Blue, appreciably less than subunits 1 and 2*, even taking into account its smaller size and greater mobility (causing greater diffusion of dye). Alternatively, the results could equally be accounted for if the genes for 1AL-LMW were merely closely linked to *Glu-A1*. As DNA sequencing of clone HMW glutenin subunit genes continues (Forde et al 1983, Thompson et al 1983) the genetic origin of 1AL-LMW may be resolved.

TABLE I
Endosperm Proteins Controlled by Chromosome 1AL in Varieties and Intervarietal Chromosome Substitution Lines

Lines/Variety	Glu-A1 Subunit	Low Molecular Weight Basic Protein
Chinese Spring	Null	Present
Cappelle-Desprez	Null	Present
Chinese Spring (Cap 1A)[a]	Null	Present
Gabota	Null	Present
Champlein	Null	Present
Koga II	Null	Absent
Hope	Subunit 1	Absent
Chinese Spring (Hope 1A)	Subunit 1	Absent
Timstein	Subunit 1	Absent
Chinese Spring (Timstein 1A)	Subunit 1	Absent
Abele	Subunit 1	Absent
Nautica	Subunit 1	Absent
Sicco	Subunit 1	Absent
Bezostaya	Subunit 2	Absent
Cap (Bezostaya 1A)[a]	Subunit 2	Absent
Lutescens	Subunit 2	Absent
Cheyenne	Subunit 2	Absent

[a]Cap is short for Cappelle-Desprez.

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