Studies of Glutenin. VI. Chromosomal Location of Genes Coding for Subunits of Glutenin of Common Wheat[1]

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ABSTRACT

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the reduced glutenins of nullisomic-tetrasomic lines of Chinese Spring wheat showed that four glutenin subunits are coded by genes on chromosome 1D. Analogous studies on ditelocentric lines showed that these genes are located on the long arm of chromosome 1D. This conclusion substantiated the fact that four subunits were absent in the durum wheat, LD222, and present in its 1D-1B substitution line. Repression of the synthesis of subunits coded by genes on chromosomes of the A or B genomes was observed in lines tetrasomic for chromosomes 2B, 3B, and 6B.

Hexaploid (bread) wheats have the genomic formula AABBDD, the A, B, and D genomes each contributing seven pairs of chromosomes. These wheats have 42 chromosomes or 21 pairs. Aneuploid lines (those that have an abnormal number or composition of chromosomes) and substitution lines (those with chromosomes of one genome substituted by homoeologous chromosomes from another) can be used to determine the chromosomal location of genes responsible for coding the synthesis of specific flour proteins.

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Particularly noteworthy in this regard are the two series of aneuploid lines of the common wheat variety Chinese Spring produced by Sears (1,2). The first series is the nullisomic-tetrasomic (nulli-tetra) lines which lack one pair of chromosomes and have a double complement of a homoeologous pair. The second series is the ditelocentrics (ditelos) in which two chromosomes of a pair lack one arm.

Shepherd (3) determined the chromosomal location of the genes that code for nine of the seventeen protein bands resolved by starch-gel electrophoresis of 2M urea extracts of single seeds of the nulli-tetras of Chinese Spring. The majority of the bands (mainly gliadins) were coded by the homoeologous chromosomes of group 1 and group 6.

Wrigley and Shepherd (4) employed a two-dimensional technique to resolve wheat gliadins into more than 40 components. The chromosomal location of genes responsible for the synthesis of more than 30 of these proteins was determined. All genes identified were located on homoeologous chromosomes of group 1 and group 6, in agreement with the findings of Shepherd (3).

Recent studies by Konarev et al. (5) showed that cytogenetic removal of the D-genome from hexaploid (common) wheat, to produce extracted tetraploid wheat, resulted in a loss of two slow-moving gliadins that can be identified by gel electrophoresis. Furthermore, it was shown that these gliadins were coded by genes on chromosome 1D.

Until the present work, there have been no reports of the chromosomal location of genes that control the synthesis of glutenin (or its subunits).

In a previous paper of this series (6) we reported that removal of the D-genome from four hexaploids resulted in the deletion of a number of glutenin subunits. Two of these subunits, identified by molecular weights of 152,000 and 112,000, are characteristic of bread wheats and are absent in all durum and extracted (AABB) tetraploid wheats studied to date. The presence of these subunits appears to be a necessary, but not sufficient, condition for breadmaking quality (6,7). Accordingly the chromosomal location of the genes that code their synthesis is of technological interest.

This article presents results on the location of genes for glutenin subunit synthesis, obtained with the two series of aneuploid lines produced by Sears (1,2) and discussed above.

MATERIALS AND METHODS

Wheat Samples

The grain of seven compensating nullisomic D-tetrasomic B (nulli D-tetra B) and the seven nulli D-tetra A lines of Chinese Spring was generously provided by E. R. Sears[3]. Each line has one pair of D-chromosomes replaced by a homoeologous pair from the B or A genome. For example, nulli 1D-tetra 1B (N1DT1B) is the line in which the 1D pair of chromosomes is replaced by an additional 1B pair. These aneuploid lines retain the normal chromosome number of 42, but the composition is abnormal.

Seven ditelocentric lines of Chinese Spring were also provided by Dr. Sears. They are 1DL, 2Dα, 3Dα, 4DL, 5DL, 6Dα, and 7DS. Ditelocentric 1DL (ditelo

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1DL) lacks the short arm of chromosome pair 1D, 2Dα lacks one of the arms (as yet not identified) of chromosome pair 2D, 7DS lacks the long arm on chromosome pair 7D.

A durum wheat (AABB), LD 222, and its 1D-1B substitution line, at the tetraploid level, were supplied by T. Mello-Sampayo[4]. The substitution line was produced by crossing Chinese Spring nulli 1B-tetra 1D to LD 222 and back-crossing to LD 222 three times with appropriate selection and selfing at each step.

Glutenin Preparation

All samples were obtained as whole grain. Approximately 0.5 g. of each line was ground with a mortar and pestle, then dispersed in the solvent AUC (0.1M acetic acid, 3M urea, and 0.01M hexadecyltrimethyl ammonium bromide) by homogenization for 5 min. Glutenins were then prepared by the pH precipitation technique described previously (8).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis was performed in a 5% polyacrylamide gel at pH 7.3, according to the method described in an earlier paper in this series (9). The gels were stained with Coomassie Brilliant Blue (C.B.B.) and were gradually destained by agitation in solutions of acetic acid-isopropanol-water (1:1:8) containing progressively decreasing amounts of C.B.B. The destained gels were stored in 10% acetic acid for subsequent examination and photography.

RESULTS AND DISCUSSION

Figure 1 shows the SDS-PAGE patterns of the reduced glutenins of Chinese Spring (C.S.) and its seven nulli D-tetra B lines. The glutenin subunits a to f are identified for reference in discussion that follows. At this point, it is of special interest to note that four of the six identified subunits, a, d, e, and f, have the same electrophoretic mobilities as the subunits previously reported to be deleted on removal of the D-genome from hexaploid bread wheats (6). Figure 1 also shows that they are all present in the parent variety, Chinese Spring. The molecular weights of these four subunits are 152,000, 112,000, 60,000, and 45,000, respectively.

Three of the four subunits, a, d, and f, are absent from the pattern of the nulli 1D-tetra 1B line. On the basis of these results, it is concluded that these three subunits are coded by genes on chromosome 1D of Chinese Spring. It is difficult to determine from the N1DT1B pattern whether the fourth subunit, e, is deleted, or whether it is diluted. If the latter is the case then there may be at least two subunits of the same molecular weight migrating together in the parent, and one is deleted. This is probably so since this subunit is not deleted in any of the other nulli D-tetra B lines.

Subunits b and c are deleted in the nulli 2D-tetra 2B, nulli 3D-tetra 3B, and nulli 6D-tetra 6B lines. These deletions are surprising since corresponding subunits were not present in the glutenin from seven accessions of *Aegilops squarrosa*, the donor of the DD-genome of bread wheats (6). Also, these subunits were not deleted in four AABB extracted tetraploids studied (6). The presence of subunit b in nulli

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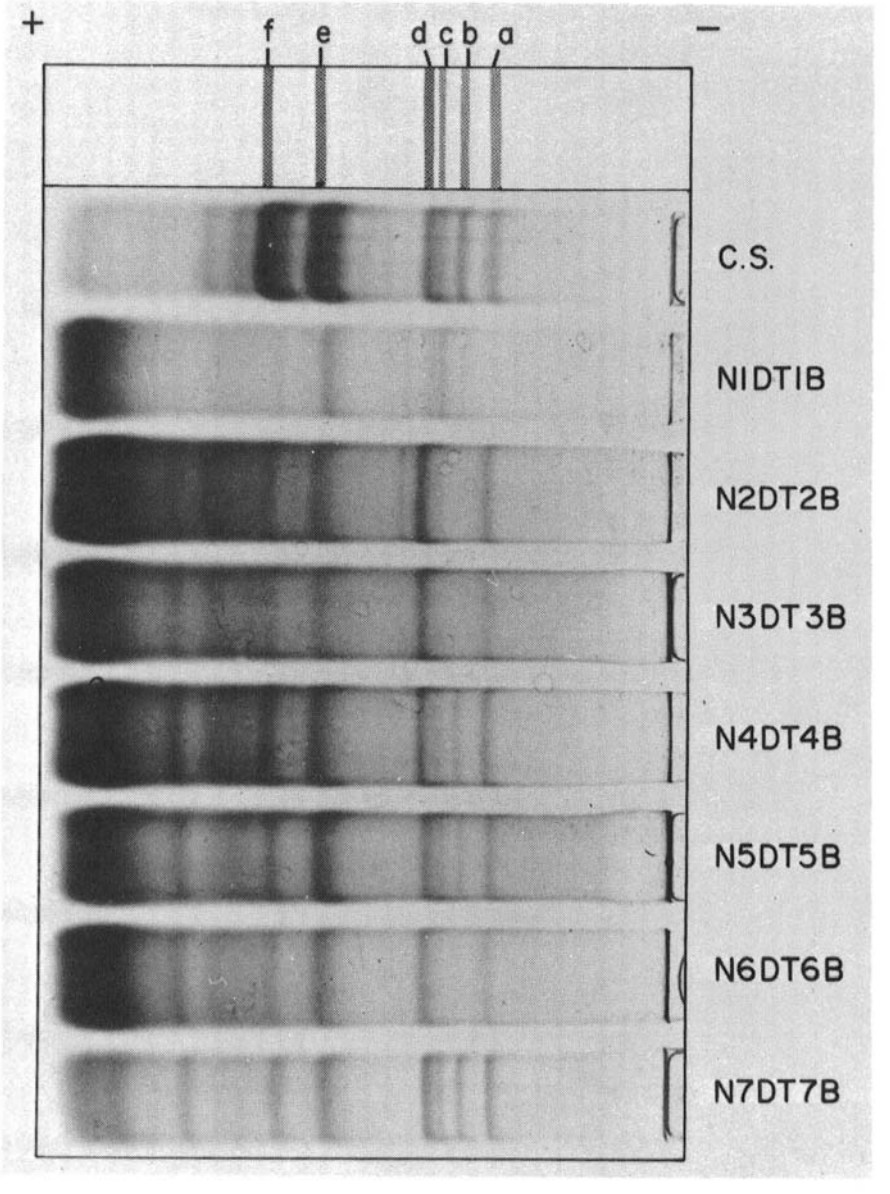

Fig. 1. SDS-PAGE patterns of reduced glutenins of the nulli D-tetra B lines of Chinese Spring.

1D-tetra 1B line (N1DT1D) was confirmed by examining the gel, although this is not clearly reproduced in Fig. 1.

There are two possible explanations of this apparent anomaly. First, the deletions could be due to removal of D-genome chromosomes coding for these

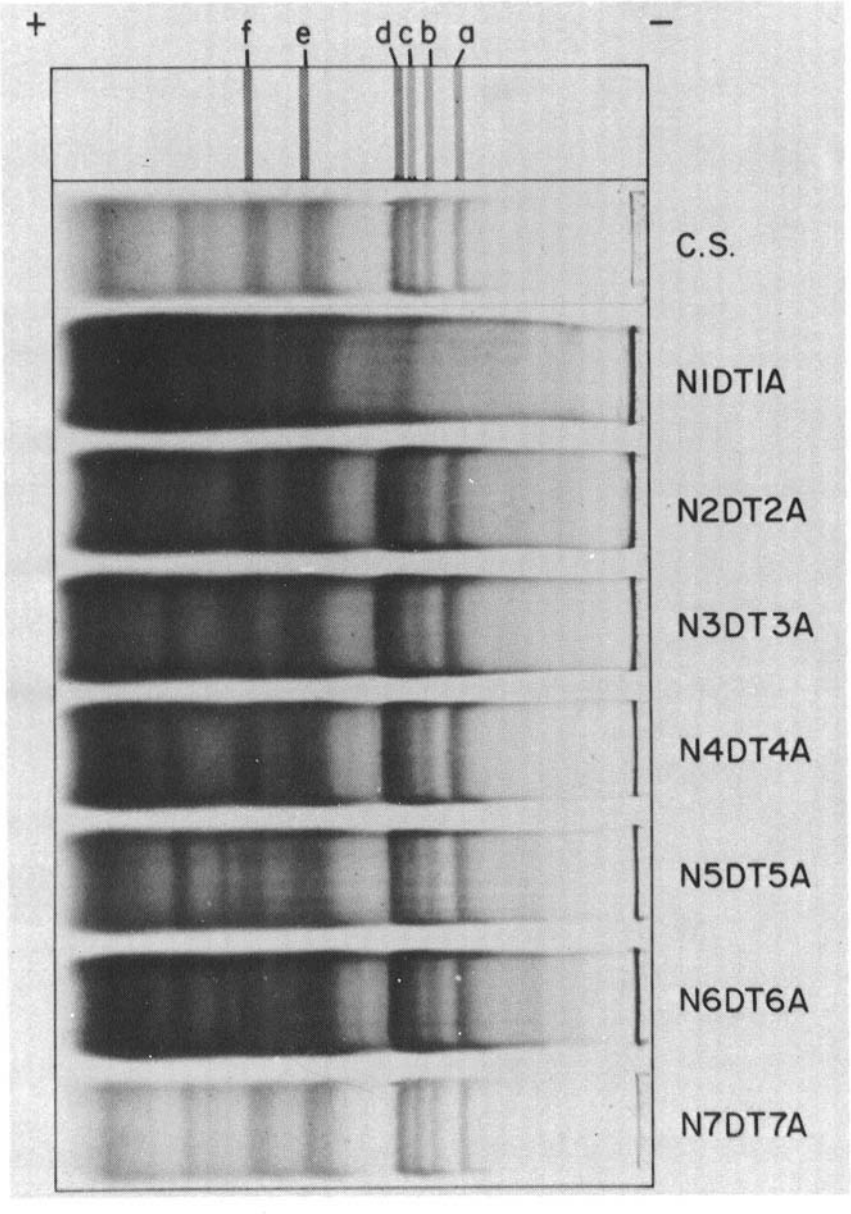

Fig. 2. SDS-PAGE patterns of reduced glutenins of the nulli D-tetra A lines of Chinese Spring.

subunits. Secondly, doubling the number of 2B, 3B, or 6B chromosomes, to achieve the tetrasomic condition, may interfere with the normal expression of genes of the A or B genomes.

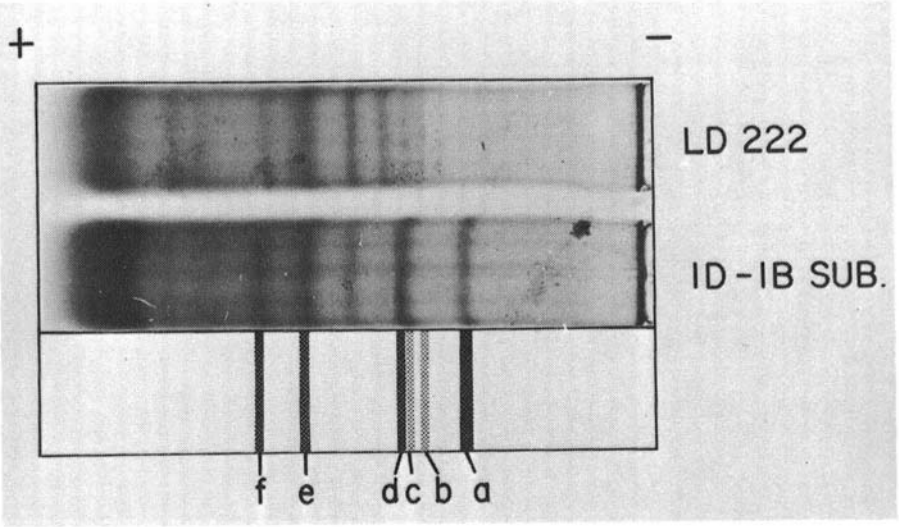

Fig. 3. SDS-PAGE patterns of reduced glutenins of a durum wheat (LD 222) and its 1D-1B substitution line (1D-1B SUB).

Results for the nulli D-tetra A lines (Fig. 2) offer a means of distinguishing between the two possibilities referred to in the previous paragraph. Once again a schematic representation of the bands under discussion is included in this figure. All lines, except nulli 1D-tetra 1A, exhibit all the bands of the parent Chinese Spring indicating that chromosomes of the D-genome do not code the synthesis of subunits b and c. This observation is consistent with the second of the two explanations—that is, the conversion of chromosomes 2B, 3B, or 6B to the tetrasomic condition has an effect on synthesis of subunits b and c. It is not known whether this repression is at the gene level or at a later stage in the biosynthesis of these polypeptides. An analogous observation was made by Shepherd (3) in the study of the 2M urea-soluble proteins of Chinese Spring and its nulli-tetra lines. When chromosome 2A was in the tetrasomic condition, the protein-forming activity of chromosome 6D was inhibited.

The absence of subunits a, d, and f in nulli 1D-tetra 1A confirms the location of their genes on chromosome 1D. Subunit e is also absent in this line, although this is not very clear from Fig. 2.

On the basis of results of Figs. 1 and 2, it can be stated with some certainty that subunits a, d, e, and f are all coded by genes on chromosome 1D. To confirm this, and to establish that the above subunits are not deleted because of the tetrasomic condition of 1A or 1B, the glutenin subunit compositions of a durum wheat (AABB) and its substitution line produced by substituting chromosome 1D of Chinese Spring for chromosome 1B of the durum, were determined. Figure 3 shows the SDS-PAGE patterns of the reduced glutenins of these two wheats with a schematic of the bands to be discussed.

If the genes for subunits a, d, e, and f of Chinese Spring are on chromosome 1D, then we would expect to find these subunits in the pattern of the substitution line.

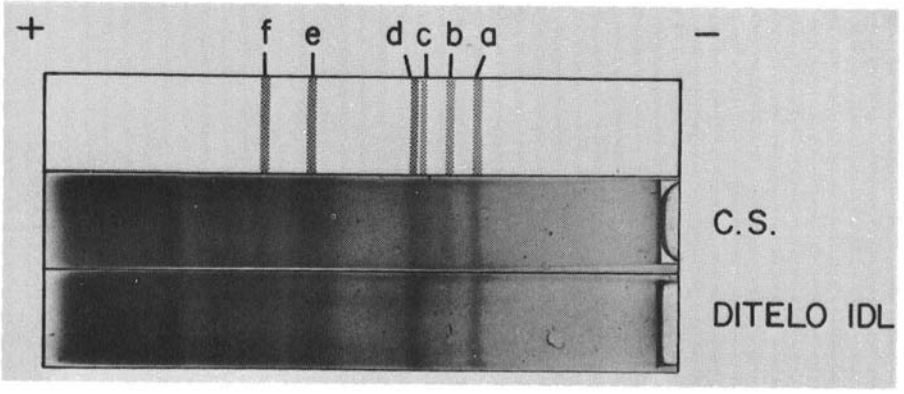

Fig. 4. SDS-PAGE patterns of reduced glutenin of Chinese Spring (C.S.) and its 1DL ditelocentric line.

Subunits a and d are obviously present in this line and absent in the durum, confirming the location of their genes on chromosome 1D.

Subunit e is in an area on the gel where an intense band also occurs in the pattern of the durum glutenin. Accordingly, it is not possible to make a definite conclusion regarding the presence of this subunit in the substitution line.

Subunit f, although close in mobility to a slightly higher-molecular-weight subunit in the durum glutenin, appears as a strong band in the substitution line.

Apart from these additional subunits caused by the presence of chromosome 1D, there are also a number of deletions. Subunits b and c are present in the durum but not in the substitution line. This indicates that they are probably coded by chromosome 1B. A similar situation exists for a subunit of slightly lower molecular weight than d (not labeled in Fig. 3). Because there is a possibility of gene repression similar to that observed in some of the nulli-tetra lines (see Fig. 1), it cannot be stated categorically that these subunits are coded by genes on chromosome 1B. There is still the possibility that the addition of chromosome 1D causes repression of the synthesis of subunits b and c. Further work is necessary to clarify this point.

Figure 4 shows the SDS-PAGE patterns of the reduced glutenin of Chinese Spring and its 1DL ditelocentric line (lacks the two short arms of chromosome 1D but has the two long arms). These patterns are qualitatively identical although there appears to be a decrease in intensity of bands b and c in the ditelocentric line. From these results, it can be concluded that subunits a, d, e, and f are coded by genes on the long arm of chromosome 1D of Chinese Spring.

GENERAL DISCUSSION

The key role of glutenin (including residue protein) in the breadmaking quality of flour is now established (10,11). Its contribution of elasticity to the rheological properties of dough is consistent with the molecular structure postulated by Huebner et al. (7) on the basis of amino acid composition of a major subunit, and with the ultrastructure of glutenin as determined by scanning electron microscopy (12). Information on the structure (all levels, primary, secondary, tertiary, and

quaternary) of glutenin is basic to the understanding of breadmaking quality at the molecular level.

A major advance in the research on the structure of glutenin has resulted from the application of SDS-PAGE to determine subunit composition (after reduction of disulfide cross-linkages). Most of the work in this area has been carried out in two laboratories: the USDA Northern Regional Laboratory in Peoria, Ill. (7,13,14), and the Department of Plant Science, University of Manitoba, Winnipeg, Canada (6,8,9). Studies of the Peoria group are aimed primarily at the fundamental structure of glutenin, whereas the Winnipeg group has concentrated on the breadmaking and genetic implications. The present article can be classified in the latter category.

It has now been established that one of the ways in which the D-genome of bread wheats affects breadmaking quality is by coding for the synthesis of at least four glutenin subunits (6). One of these is the largest and another the fourth largest that have so far been identified. It appears that these subunits play a key role in the structure of glutenin that is essential for breadmaking quality; that they are absent in durum wheats is one of the reasons these wheats lack breadmaking quality (6). Note that these subunits are not the total answer to breadmaking quality (6,9); there are many other factors, some dependent on flour proteins and some on other constitutents (lipids, pentosans, starch).

The mechanism by which the two subunits (largest and fourth largest) affect the structure of glutenin (and thereby gluten and dough) has not been established. They could occupy a central position in the linking, through covalent bands, of other subunits into the large glutenin molecule(s). Alternatively, because of glutenin's fibrous structure, the key role of the subunits might depend on secondary (hydrogen bonds and hydrophobic interactions) bonds. Amino acid composition data of the largest glutenin subunit from a bread wheat (7) and scanning electron microscopy results on glutenin (12) are consistent with the second mechanism. In relation to this statement, it is now known that fibrous proteins such as collagen and keratin are composed of helical subunits with extensive inter- and intramolecular secondary interactions.

The present work establishes the location of the genes for some of the glutenin subunits identified with breadmaking quality as the long arm of chromosome 1D in Chinese Spring. If this location is general for bread wheats, or the alternate location can be determined, it would be extremely useful to cytogeneticists. In developing synthetic cereal species such as triticale, or in attempting to add (cytogenetically) breadmaking quality to durum (tetraploid) wheats, cytogeneticists should pay special attention to the inclusion in their progeny of the D-genome chromosome(s) responsible for the synthesis of these important glutenin subunits.

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