Two-dimensional analysis of gliadin proteins associated with quality in durum wheat: chromosomal location of genes for their synthesis

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Summary. Two-dimensional electrophoresis was used to fractionate the gliadin proteins from the endosperm of durum wheat. The increased resolution of the system, as compared with single-dimensional analysis, accentuated the heterogeneity of the proteins. This resolution, coupled with the use of aneuploid lines of the cultivar 'Langdon', permitted identification of the chromosomes controlling synthesis of the major protein components. Homoeologous Group 1 chromosomes controlled omega- and gamma-gliadin synthesis and the Group 6 chromosomes 6A and 6B controlled alpha- and beta-gliadins. Chromosome 1B was primarily responsible for the two groups of protein-polypeptides associated with strong or weak gluten characteristics of durum wheat. However, some of these proteins were controlled by chromosome 1A. In the beta-gliadin region several hybrid bands, whose chromosomal control was not identified by electrophoresis alone, were specified primarily by genes on chromosome 6B, although chromosome 6A was also involved. Control of some other hybrid bands could not be determined. Chromosomes in Groups 2, 3, 4, 5 and 7 were not implicated in the synthesis of the gliadin proteins of durum wheat.

Key words: Triticum turgidum L. group durum — Gliadin — Chromosome — Two-dimensional electrophoresis

Introduction

Evidence is accumulating of a close relationship between the presence of a specific gliadin protein, designated band 45 on its electrophoretic mobility, and strong gluten in durum wheat, (Triticum turgidum L. group durum). Another protein (band 42) appears to be associated with weak viscoelastic properties (Damidaux et al. 1978, 1980a; Kosmolak et al. 1980). These results were extended by the demonstration that, in over 100 durum genotypes, a group of gliadins (group 45), not just one protein, was associated with strong dough characteristics and another group (group 42) with dough weakness (du Cros et al. 1982).

Damidaux et al. (1980b) also found close associations between a few gliadins and band 42 and between one other gliadin and band 45, and attempted to establish the chromosomal location of genes controlling their synthesis. They reported that the proteins associated with gliadin 42, as well as gliadin 42 itself, were coded for by genes on chromosome 1B. Joppa et al. (1983) performed polyacrylamide gel electrophoresis (PAGE) on gliadin proteins from the endosperm of a set of 'Langdon' durum disomic-substitutions and determined the chromosomal location of genes coding for many of these polypeptides. However, the chromosomal location of genes coding for some gliadin-polypeptides could not be determined because the proteins could not be separated by the methods used and some bands appeared to be controlled by more than one chromosome. One of these bands is associated with gliadin 42 (du Cros et al. 1982). Aneuploids of durum wheat possessing the group-45 proteins are not presently available. However, Damidaux et al. (1980b) reported that band 42 and band 45 appeared to be controlled by alleles at the same locus, or at closely linked loci. Joppa et al. (1983) substituted the 1B chromosome from 'Edmore', a strong gluten cultivar (with band 45), into 'Langdon', a cultivar having poor dough strength (and band 42), and established that both band 45 and band 42 are controlled by genes on chromosome 1B.
Thus, single-dimension PAGE has proved valuable in the identification of chromosomes controlling the synthesis of certain gliadin proteins. This system, however, is limited to identification of cases where a chromosome has sole control over the synthesis of a particular band. Difficulties arise when two, or more, chromosomes are involved in the production of a single-dimension protein band. Two-dimensional electrophoresis provides greatly improved resolution of these protein components. The system generally involves isoelectric focusing in the first dimension, where the proteins are separated according to their overall charge. This is followed by either PAGE or sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension, where the proteins are separated on the basis of charge and size or size alone. Thus, protein bands, which appear to be single polypeptides in one dimension but which are actually composed of two or more proteins, will be resolved into their component polypeptides in the two-dimensional system. This type of analysis was used in aneuploid studies of hexaploid wheat (Wrigley 1970; Wrigley and Shepherd 1973; Brown and Flavell 1981), and has clarified interpretation of the results in a way not possible by electrophoresis in one dimension.

In the study reported here, we have used two-dimensional electrophoresis to examine the chromosomal location of genes coding for proteins, particularly those associated with poor quality, in durum wheat. This resulted in better resolution of the gliadin proteins, partial characterization according to isoelectric properties, and consequently improved understanding of the relevant gene locations.

Materials and methods

Grain samples

The Australian cultivar 'Durati', representing a gliadin band 45 durum wheat (45-type), and the cultivar 'Leeds' (42-type), from the USA were examined to determine the complexity of the proteins associated with quality. Other cultivars studied were 'Langdon' durum (42-type with weak gluten) and the hexaploid wheat 'Chinese Spring'.

Chromosomal locations of genes coding for the quality-related proteins were studied using a set of 'Langdon' disomic substitution lines in which individual D-genome chromosomes from 'Chinese Spring' replace their A- or B-genome homoeologues (Joppa et al. 1978, 1983). The disomic-nullisomic combinations 4D(4B) and 7D(7A) were not available for examination. A line, in which chromosome 1B from 'Edmore' (a 45-type durum with strong gluten) was substituted into 'Langdon' by crossing and backcrossing to the 'Langdon' 1D(1B) line, was also examined.

Cytology

Several of the substitution lines must be maintained by carrying one of the substituted 'Langdon' chromosomes in the monosomic or telosomic condition. Hence the appropriate plants with $2n=28$ (and presumed to represent 14 chromosome pairs) were identified by somatic chromosome counts on root tips. Kernels from lines being tested were cut in halves, the distal portions reserved for electrophoresis, and the embryo halves germinated. Somatic chromosome numbers were determined using the Feulgen-acetocarmine squash technique. After germination, root-tips were collected and placed in ice-water for 24 h. The root-tips were fixed in alcohol: acetic acid (3:1) solution for 24 h and then hydrolysed in 1N HCl in a water bath for 11.5 min at 56°C. After staining in leuco-basic Fuchsin for at least 20 min the root tips were squashed in acetocarmine and examined under the light microscope. Only half-portions from those kernels containing embryos with $2n=28$ were examined electrophoretically.

Extraction and electrophoresis

The distal halves of selected kernels were crushed and the gliadin proteins extracted overnight at 25 °C in 6% urea (6 ml/ mg grain). For examination of cultivars, ground wholemeal samples were extracted.

Single-dimensional PAGE of cultivars was carried out, with slight modifications to the gels, as described below for electrophoresis in the second-dimension. The gel slabs measured approximately 140x50x1 mm and were cast with a polyacrylamide gradient ranging from 3% at the top to 15% at the bottom; sample slots were formed using a 3% gel.

a) Isoelectric focusing. Glass tubes (70 mm long; 3 mm i.d.) were filled to 60 mm with 6% polyacrylamide solution containing 6% urea and 0.5% carrier ampholytes (Pharmacia AB, Uppsala, Sweden) in the range pH 5 to pH 9. The pH gradient was prefocused for 30 min at 400 V with 0.2% sulphuric acid at the upper electrode (anode) and 0.4% ethanalamine at the lower electrode (cathode). The protein extracts were clarified by centrifugation at 7,500 g for 15 min. 50–60 µl of each extract applied and electrofocusing was continued for 2.5 h at 400 V. The gels were rimmed from their glass tubes and immediately used for protein separation in the second dimension.

b) Gradient gel preparation and electrophoresis. The polyacrylamide gel slabs (165x135x3 mm) were cast in tailed glass cassettes using a multichannel peristaltic pump and an equal quantity (90 ml) of two solutions with acrylamide concentrations of 3% and 11%, respectively. Each solution contained sodium lactate buffer (0.004 M Na, pH 3.1), 0.04% N,N,N',N'-tetramethylethylenediamine (TEMED), and 0.004% ammonium persulphate. Two gels were cast simultaneously in a gel slab-casting apparatus using pump tubes of (a) 2 ml/min and (b) 4 ml/min capacity. To ensure polymerization and a flat gel top, a small amount of diluted buffer (1:10) was initially pumped into the apparatus. The higher concentration acrylamide solution was then pumped via tube (a) to the low concentration solution, which was stirred continuously. At the same time this solution was pumped through tube (b) to the bottom of the casting apparatus to form the gradient. After all of the acrylamide solution had been pumped into the apparatus, 20% glycerol solution was pumped in to bring the acrylamide level to the bottom of the gel cassettes. At this stage the overlaying buffer was near the top of the cassettes. Total time for pumping was about 1 h. Polymerization of the gels occurred soon after casting was complete. Gels were always prepared one day before use to ensure complete destruction of unreacted free radicals from the polymerisation reaction.

Electrophoresis in the second dimension was performed in sodium lactate solution (pH 3.1, 0.004 M Na). Two slab gels in