Changes in Protein Complexes of Durum Wheat in Developing Seed

Florence Bénétrix, François Kaan, and Jean-Claude Autran*
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ABSTRACT
End-use quality of wheat derives from the functional properties of its storage proteins. Storage proteins are synthesized during grain development and undergo modifications mainly during grain dehydration, with increased level of aggregation. This study was conducted to determine whether changes in protein complexes of durum wheat [Triticum turgidum (Desf.)] during seed development relate to seed quality. Protein extracts of developing seeds of cultivars Capdur and Tomclair were reduced and examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Results confirmed synthesis of both gliadins and glutenin subunits early during maturation with qualitative compositions remaining nearly constant. Size-exclusion high-performance liquid chromatography (SE-HPLC) on unreduced protein extracts was used to follow quantitative changes in distribution of protein complexes. Among the five chromatographic fractions corresponding to different sizes of aggregates or monomers, F1 (excluded peak) and F2 (complexes of intermediate size) increased more rapidly in Capdur (good pasta quality) than in Tomclair (poor pasta quality). Characterization of the chromatographic fractions by electrophoresis showed that low-molecular-weight (LMW) subunits of glutenin are mainly involved in the largest complexes and are the ones most clearly showing differences between cultivars. The tendency of LMW subunits to aggregate during grain dehydration may help explain differences found in pasta quality among durum wheat cultivars.

Durum wheat is widely considered the best wheat for pasta products because of its excellent amber color and superior cooking quality. Differences in cooking quality among wheats are attributed to the protein content and composition of the grain endosperm (Feillet, 1977; 1988).

Although the classical Osborne's scheme (Osborne, 1907) for protein fractionation continues to be widely used, other approaches have been suggested. For instance, Miftin et al. (1983) and Shewry et al. (1984) proposed a nomenclature related to functional properties of wheat storage proteins, including S-poor proteins (α- and β-gliadins), S-rich proteins (α- and β-gliadins), and LMW and high-molecular-weight (HMW) subunits of glutenin.

A major breakthrough in understanding the genetic basis of durum wheat quality was realized by Damidaux et al. (1978) with the discovery of a relationship between the electrophoretic patterns of γ-gliadins and gluten strength. Allelic type γ–45 was associated with a strong gluten, whereas allelic type γ–42 was associated with a weak gluten. In fact, the positive effect of γ-gliadin 45 originated from aggregative subunits of the Glu-B3 locus, genetically linked with the Gli-B1 locus (Pogna et al., 1988). Recently, significant correlation was shown between pasta quality potential and the ratio of aggregative fractions, especially LMW subunits of glutenin, in the endosperm, whereas other aggregative fractions, such as HMW glutenin subunits, played a less significant role in the differences in gluten quality amongst durum wheat genotypes (Autran and Galterio, 1989). Durum wheats must be bred for high quality potential and stability of quality expression; however, the latter is poorly understood and cannot be predicted by breeders.

To investigate mechanisms of expression of seed quality, a relevant approach is to study accumulation of storage proteins in the wheat kernel during grain development. Results in this field are contradictory, however, mainly because different techniques and extracting agents have been used. For instance, according to Reeves et al. (1986), all storage proteins are synthesized from 12 to 15 d post-anthesis. In contrast, Skerritt et al. (1988) reported a chronological accumulation of gliadins, a few days after the glutenin synthesis. Because technological quality of wheats is primarily determined by the occurrence of large protein aggregates and the size and composition of these protein aggregates are influenced by environmental conditions (Kaczkowski et al., 1987), to only study protein monomers or reduced subunits by electrophoresis is unlikely to give insights into mechanisms of expression of quality during grain development.

The recent introduction of SE-HPLC in the study of bread-wheat protein aggregates allowed more accurate determination of the size range of unreduced protein complexes than did solubility methods (Autran, 1994) and allowed identification of various indicators of baking quality (Dachkevitch and Autran, 1989). In the same way, Millet et al. (1991) showed that the distribution of protein aggregates in the barley (Hordeum vulgare L.) kernel was related to malting quality.

In the present study, SE-HPLC and SDS-PAGE were used to investigate changes in protein complexes to relate expression of quality with mechanisms of protein aggregation. Analyses were carried out on kernels sampled during grain development from two durum wheat cultivars, each representing a quality type.

MATERIALS AND METHODS

Wheat Samples. The two French durum wheat cultivars selected were Capdur, an excellent pasta-making-quality wheat, and Tomclair, a very poor pasta-making-quality wheat.

Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SE-HPLC, size-exclusion high performance liquid chromatography; LMW, low molecular weight; HMW, high molecular weight; F1, soluble fraction; F2, insoluble fraction; dmt, daily mean temperature.
Plants were grown in 1989 at the Montpellier INRA experimental field (Montpellier, France). At anthesis (10–16 May), heads were labelled and then sampled at 2- to 3-d intervals until fully ripe. Nighttime and daytime temperature ranges were recorded daily. Maximum temperatures ranged from 17.3 to 27.9 °C in May and from 20.3 to 34.0 °C in June. Excised heads were frozen at −20 °C, freeze-dried, and threshed. Dry seeds were finely ground in a Tecator Cyclotec laboratory mill (Tripette et Renaud, Paris) and stored at 4 °C. Because temperature affects the formation of grain storage components, results are reported as a function of the cumulative daily mean temperature after flowering (Cerning and Guilbot, 1973).

Protein Extraction. To be analyzed by SE-HPLC, proteins were extracted from ground seeds (80 mg) by 10 mL of 0.1 M sodium phosphate buffer (pH 6.9) containing 2% sodium dodecyl sulfate (SDS) for 2 h at 60 °C. Extractions were followed by centrifugation for 30 min at 37 500 × g at 20 °C. The amount of proteins extractable by the phosphate-SDS buffer was defined as the soluble fraction (Fs), expressed on a percentage of total protein basis. The percentage of insoluble fraction (Fi) was determined by Kjeldahl analysis of the residues (%Fi = total protein content) as in Dachkevitch (1989).

SE-HPLC. Instrumentation for SE-HPLC was described previously (Dachkevitch and Autran, 1989). A Beckman (Carlsbad, CA) TSK4000 SW size-exclusion analytical column (7.5 by 300 mm, 450 Å) protected by a guard column (7.5 by 75 mm, 250 Å) was used. A 0.1 M sodium phosphate buffer (pH 6.9) containing 0.1% SDS was used as eluent with a flow rate of 0.7 mL/min. Twenty microliters of supernatant were applied to the column using an automated sample injector. The column effluent was monitored at 214 nm and 0.1 absorbance units full scale. The chromatograms were analyzed through Nelson analytical software, which permitted integration of the elution curve. Reproducibility of the method, previously investigated by Dachkevitch and Autran (1989), was sufficient to observe small differences between samples. For instance, coefficients of variation of percentages were 1 to 2% when loading the same protein extract several times and were 2 to 3% when running different protein extracts of the same flour.

Electrophoresis. Total proteins were extracted by a reducing solvent [1 M tris/HCl buffer (2-amino-2-hydroxymethyl-1,3-propanediol), pH 6.8, 20 g L⁻¹ SDS, 100 mL L⁻¹ glycerol, 0.1 g L⁻¹ pyronin (3,6-Bis(dimethylamino)xanthylum chloride), and 50 mL L⁻¹ 2-mercaptoethanol] and fractionated by 13% SDS-PAGE according to Autran and Berrier (1984). Subunit composition of SE-HPLC peaks was also determined by SDS-PAGE. To recover concentrated proteins from collected peaks, the SDS was removed by precipitating the protein fraction by 150 g L⁻¹ trichloroacetic acid according to Dachkevitch (1989). The protein residue was washed two times with 1 mL of acetone and air dried. Dry protein extracts were dissolved in the Tris-SDS reducing buffer, then electrophoresed by 13% SDS-PAGE. Percentage of the main bands or groups of bands was estimated by densitometric scanning with a soft laser densitometer (Ultrascan 2002, LKB Instruments, Bromma, Sweden) according to Autran et al. (1987). Because fast- and slow-moving fractions have different amino acid compositions and are likely to bind different proportions of Coomasie Blue, it is well known that the recorded band intensities may not reflect true amounts of the various protein factions. However, intra-gel comparisons of homologous bands were shown to yield reliable and reproducible (±2%) results (Mecham et al., 1981; Autran et al., 1987).

Nitrogen Determination. Nitrogen content of the ground seeds and extracts was determined by Kjeldahl analysis using a Cu–Se catalyst.

RESULTS AND DISCUSSION
Changes in Electrophoretic Patterns. The SDS-PAGE patterns of total reduced protein were determined for 15 samples of developing kernels for each cultivar, ranging from Σ daily mean temperature (dmt) 200 °C to maturity (Fig. 2). All main protein fractions of mature kernels were present at the earliest stages of kernel development. Patterns remained essentially stable from the earliest stages of development until maturity, except...
for some variation in band intensities. Such a lack of major changes in the electrophoretic patterns is consistent with most results published concerning bread wheat (Feillet, 1965; Bushuk and Wrigley, 1971; Mecham et al., 1981; Tercé-Laforgue and Pernollet, 1982; Huebner et al., 1990) or durum wheat (Galterio et al., 1987).

We attempted to further investigate quantitative variation of various protein fractions [HMW and LMW subunits of glutenin, main (α + β + γ) gliadins, ω-gliadin, and albumins] through accurate densitometric scanning of the patterns (Table 1). Observed trends confirmed previous reports, namely (i) a rapid increase of gliadins until a maximum at Σdmt 550°C (i.e., about 4 wk post-anthesis), (ii) a later synthesis of glutenin subunits, and (iii) a decrease of albumins. Although most temperatures more than 30°C were recorded after mid-June, during the dehydration step, some changes observed in relative amounts of gliadin and glutenin fractions may

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Fig. 2. SDS-PAGE patterns of reduced protein extracts from seeds of Capdur (a) and Tomclair (b) at different stages of development. Σdmt °C are indicated. R = mature seeds. MW = molecular weight reference mixture. Molecular weight markers are phosphorylase b, 94 000; bovine serum albumin, 68 000; ovalbumin, 43 000; carbonic anhydrase, 30 000; trypsin inhibitor, 20 100; α-lactalbumine, 14 400. Major polypeptides are indicated on the left.
Table 1. Changes in distribution of relative amount of main protein fractions [high (HMW) and low (LMW) molecular weight subunits of glutenin, ω-gliadin, albumins, and (α + β + γ) gliadins] at four developmental stages (Σdmt°C = 200, 410, 610, and mature seed) in endosperm samples from durum wheat cultivars Tomclair and Capdur. Total amounts do not reach 100% because only data on the most typical fractions are given.

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>HMW glutenins</th>
<th>LMW glutenins</th>
<th>ω-gliadin</th>
<th>Albumins</th>
<th>(α + β + γ) gliadins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capdur</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σdmt 200 °C</td>
<td>0.6</td>
<td>12.2</td>
<td>1.8</td>
<td>38.7</td>
<td>30.1</td>
</tr>
<tr>
<td>Σdmt 410 °C</td>
<td>1.2</td>
<td>16.4</td>
<td>1.9</td>
<td>35.8</td>
<td>31.7</td>
</tr>
<tr>
<td>Σdmt 610 °C</td>
<td>1.9</td>
<td>17.9</td>
<td>2.7</td>
<td>32.9</td>
<td>33.5</td>
</tr>
<tr>
<td>Mature seed</td>
<td>1.7</td>
<td>24.4</td>
<td>1.7</td>
<td>32.3</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td>Tomclair</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σdmt 200 °C</td>
<td>0.3</td>
<td>22.3</td>
<td>2.2</td>
<td>23.7</td>
<td>41.5</td>
</tr>
<tr>
<td>Σdmt 410 °C</td>
<td>0.5</td>
<td>22.0</td>
<td>2.4</td>
<td>23.5</td>
<td>41.0</td>
</tr>
<tr>
<td>Σdmt 610 °C</td>
<td>1.2</td>
<td>25.5</td>
<td>3.5</td>
<td>25.3</td>
<td>35.7</td>
</tr>
<tr>
<td>Mature seed</td>
<td>1.3</td>
<td>26.5</td>
<td>2.0</td>
<td>24.4</td>
<td>37.2</td>
</tr>
</tbody>
</table>

have been the result of high temperatures (Blumenthal et al., 1990). However, no clear difference in the biosynthetic trend between the two cultivars was observed.

Chromatographic Fractions. The SE-HPLC profiles of unreduced proteins extracted with sodium phosphate-SDS buffer differed among the four developmental stages of each cultivar (Fig. 3). The magnitude of the excluded peak (F1) progressively increased for both varieties as the seeds developed, but this increase was steadier and more obvious for Capdur (Fig. 4). The percentage of Fraction F2 (intermediate aggregates) remained essentially unchanged in Tomclair (~15%), whereas in Capdur, F2 increased very rapidly from 11 to 21% at about Σdmt 600°C, reaching a constant value (~20%) during grain dehydration. Because large changes occurred in F2, this fraction was split into F2a (mol. wt. 250 000–600 000) and F2b (mol. wt. 100 000–250 000).

Fig. 3. Changes in SE-HPLC protein elution profiles (unreduced) from Capdur (a) and Tomclair (b) at three stages of development: (1) Σdmt °C = 200, (2) = 410, and (3) = 610 and from mature seed (4).

Fig. 4. Changes in percentages of some SE-HPLC fractions upon grain development and maturation from Capdur (a) and Tomclair (b), F1 (mol. wt. > 600 000); F2 (mol. wt. 100 000–600 000); F2a (mol. wt. 250 000–600 000); F2b (mol. wt. 100 000–250 000); F4 (mol. wt. 20 000–55 000).
For Capdur, before $\Sigma_{dm} 650^\circ C$, proteins of molecular weight lower than 250,000 (F2b) were present in larger amounts than those higher molecular weight (F2a). During grain dehydration, an inversion of the slope occurred in chromatograms, indicating that Fraction F2a became predominant. In contrast, no inversion of slope was observed with Tomclair; F2a and F2b tended to be constant after $\Sigma_{dm} 700^\circ C$.

The main types of monomers (mol. wt. 20,000–50,000) (Fraction F4) tended to increase in Tomclair during grain maturation, whereas in Capdur, this fraction decreased slightly in early stages of development and then remained essentially unchanged. In addition, because a portion of the highest molecular weight protein (glutenin) usually remains insoluble regardless of extraction conditions, changes in nitrogen $Fi$ were followed by Kjeldahl analysis (Dachkevitch, 1989).

The $Fi$ increased in Capdur but remained essentially constant in Tomclair (Fig. 5). During grain drying, Capdur, which produces a strong gluten, also had greater amounts of insoluble protein than did Tomclair, which produces a weaker gluten. This agrees with results for bread wheats obtained by Lundh and MacRitchie (1989).

These results suggest that monomers or LMW aggregates were transformed into higher molecular weight complexes during kernel development. These changes were greater in Capdur than in Tomclair. Increased protein aggregation and insolubilization during development and dehydration appeared greater in high-quality than in low-quality cultivars. This conclusion is supported by opposite trends in (i) amount of $Fi$, (ii) percentage of excluded peak (F1), (iii) percentage of monomeric fractions (F4), and (iv) inversion of slope in the profile of intermediate aggregates (F2).

Protein Characterization. Compositions of protein in the nine fractions collected from the Superose-6 gel-filtration column were analyzed by SDS-PAGE with reduction by 2-mercaptoethanol in comparison with the whole phosphate-SDS extract and with the total reduced proteins (Fig. 6). The first SE-HPLC fraction (corresponding to the first region of the elution curve, i.e., excluded peak F1) consisted essentially of HMW and LMW subunits of glutenin, whereas LMW glutenin subunits predominated in the next three fractions that made up the intermediate aggregates (F2). Small amounts of HMW glutenin subunits and gliadins were also present in F2. The two fractions of Peak F3 contained LMW glutenin subunits, $\alpha$-gliadins, and a large amount of $\alpha$-, $\beta$-, and $\gamma$-gliadins as well as some other fast-moving gliadins and albumins. Peak F4 contained only $\alpha$, $\beta$, and $\gamma$ types of gliadins with some fast-moving proteins.
and Peak F5 was made up of some fast-moving gliadins and albumins with still some α-, β-, and γ-gliadins eluting later. These compositions of the main SE-HPLC peaks corroborate previous results of Dachkevitch (1989) on bread wheats and especially the presence of LMW subunits in intermediate-sized aggregates F2 (100,000–600,000). Our results emphasize the contribution of LMW subunits because Subfractions F2a and F2b (Fig. 4) were most clearly involved in changes in protein composition during grain development and dehydration. Likewise, LMW subunits were the ones most clearly showing differences between cultivars, indicating that these subunits may be major indicators of quality among durum wheats.

CONCLUSIONS

It appears that SE-HPLC is a powerful tool for studying native protein aggregates, providing information not available with electrophoresis techniques. With SE-HPLC, we found that strong modifications occur mainly during grain dehydration, with increased level of aggregation.

To our knowledge, this study is the first investigation of changes in protein complexes of durum wheat during seed development as they relate to seed quality. Our results provide a basis for further investigations of aggregate composition of wheat proteins in developing seed.

In our results with two contrasting cultivars, several trends occurred:

1. Molecular size distributions of storage proteins changed throughout grain development, especially during grain dehydration. The trend was for increased size of protein aggregates.

2. There are noticeable differences in accumulation of large aggregates (insoluble residue and Fraction F1), medium-sized aggregates (Fraction F2), and monomers (e.g., Fraction F4) between the two cultivars. For instance, increases in F1, F2, and the insoluble residue were more apparent in Capdur than in Tomclair.

3. As suggested by inversion of the slope in the F2 region [i.e., the changes in the respective abundance of protein types F2a (mol. wt. 250,000–600,000) and F2b (mol. wt. 100,000–250,000) during development], the aggregation phenomenon largely involves LMW subunits of glutenin. The presence and proportion on the basis of total proteins of the LMW subunits of glutenin have been clearly associated with intrinsic pasta quality of durum wheat genotypes (Autran et al., 1987; Feillet et al., 1989). We postulate that the tendency of LMW subunits of glutenin to aggregate during grain dehydration may help explain differences found in pasta quality among durum wheat cultivars.

Moreover, because it has been reported that the percentage of gliadins (Fraction F4) is greatly influenced by environment, it is likely that aggregation profiles, inferred from SE-HPLC, may reveal environmental effects within cultivars. Unlike SDS-PAGE, which primarily gives fingerprints of genotypes, SE-HPLC may help explain quality differences of cultivars grown under various climates and agricultural practices and may be of interest to many agronomists and chemists.

REFERENCES


