Identification of a Unique Group of High Molecular Weight Proteins in Some Wheat Varieties[1]

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ABSTRACT

A unique group of high molecular weight proteins, similar in mobility to the high molecular weight subunits of glutenin, was identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in some Canadian wheat varieties. These proteins are extractable from flour or gluten with acetic acid and with acetic acid/urea solutions. They were not extracted with traditional solvents used for glutenin extraction that contain detergents such as cetyltrimethylammonium bromide or sodium dodecyl sulfate/2-mercaptoethanol.

Since the finding by Orth and Bushuk (1972) of the highly significant correlation between the solubility of wheat glutenin in dilute acetic acid solution and breadmaking quality, there has been considerable research on a possible relationship between the structure of glutenin and its functionality in breadmaking. In particular, many workers have shown an association between the composition of the high molecular weight (HMW) glutenin subunits and breadmaking potential of wheat varieties (Branlard and Dardevet 1985, Burnouf and Bouriquet 1980, Campbell et al 1987, Lawrence et al 1987, Ng and Bushuk 1988, Payne et al 1979, Payne et al 1987). Through the outstanding efforts of Payne and co-workers and others (Payne et al 1980, Payne et al 1981, Holt et al 1981, Lawrence and Shepherd 1980), more than 20 HMW glutenin subunits have been identified and genetically classified in common wheat varieties. This paper reports the presence in some Canadian wheat varieties of additional HMW proteins that, despite their extractability from flour or gluten with acetic acid and acetic acid/urea solutions, have apparently remained until now undetected, probably because they are not extractable by commonly used glutenin solvents that contain detergents such as cetyltrimethylammonium bromide (CTMAB) or sodium dodecyl sulfate (SDS).

MATERIALS AND METHODS

Samples

Grain of four Canadian hard red spring wheat cultivars (Neepawa, Katepwa, Marquis, Roblin), one soft white spring cultivar (Fielder), and one Canada Prairie Spring cultivar (HY320) was used.

Flour

Flour was milled on a Buhler experimental mill. Small quantities of whole grain meal of varieties Marquis, Fielder, and HY320 were prepared by crushing the grain with a hammer and sifting through a 0.4-mm mesh sieve.

Gluten

Gluten was prepared from Neepawa flour by hand washing using distilled water.

Protein Extractions

Prior to the use of different solvents for extraction of the HMW proteins, the flour (500 mg, dm) and gluten (100 mg, dm) samples were extracted with 20 ml of 70% ethanol to remove the ethanol-soluble proteins (gliadins) and thereby decrease the amount of protein that was eventually loaded onto the electrophoresis gel. Initial extraction with 0.5M NaCl had no effect on the SDS-polyacrylamide gel electrophoresis (PAGE) pattern of the HMW subunits (data not shown); accordingly it was omitted in order to minimize the extraction time. The ethanol extracts were separated by centrifugation at 12,000 $\times$ g at 4°C for 5 min and decantation. The residue was then extracted with the following solvents: 1) 0.1M acetic acid, 2) 3M urea, 3) 0.01M CTMAB, 4) 0.1M acetic acid + 3M urea (AU), 5) 3M urea + 0.01M CTMAB (UC), and 6) 0.1M acetic acid + 3M urea + 0.01M CTMAB (AUC). Extraction was for 2 hr at room temperature in 20 ml of solvent in 30-ml glass centrifuge tubes under gentle shaking. After each extraction, the protein solution obtained by centrifugation (10 min at 12,000 $\times$ g at 4°C) was dialyzed against double-distilled water at 2°C for three days with five changes. The dialyzed extracts were freeze-dried.

Electrophoresis

SDS-PAGE was carried out as described by Ng and Bushuk (1987). Flour (40 mg), 10 mg of freeze-dried gluten, or 5 mg of freeze-dried protein extract was treated with 1 ml of solvent containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 60 mM Tris-HCl, and 0.01% pyronin Y (pH 6.8). Each mixture was allowed to stand for 2 hr at room temperature and then heated for 3 min in a boiling water bath. The samples were allowed to settle at room temperature for half an hour, and 7-10-μl aliquots of the supernatant were loaded onto the SDS-PAGE gels.

RESULTS AND DISCUSSION

High Molecular Weight Proteins of Neepawa Wheat

Figure 1 shows the SDS-PAGE patterns of different extracts of the wheat variety Neepawa. By comparison of corresponding lanes, it can be seen clearly that the pattern for the AU extract (lane f) contains a distinct additional band (K1) with mobility lower than that of HMW subunit 9 of glutenin (the subunits are numbered according to the Payne and Lawrence [1983] nomenclature as described by Ng and Bushuk [1988]). The additional band was also detectable as a faint band in the pattern for the protein extracted by acetic acid alone (lane c). However, it was absent in the patterns of extracts with UC (lane g) and AUC (lane h) solvents or in the patterns for flour (lane a) and gluten (lane b) obtained by the direct SDS-PAGE analysis. When the flour was preextracted with salt solution and/or ethanol solution, according to the modified Osborne procedure (Chen and Bushuk 1970), we found that in neither of these solvents was the K1 band soluble (data not shown). However, we used the preextraction with ethanol to reduce the amount of protein that was applied to the gels in electrophoresis.

In the original report on the use of AUC to solubilize wheat storage proteins, Meredith and Wren (1966) observed that

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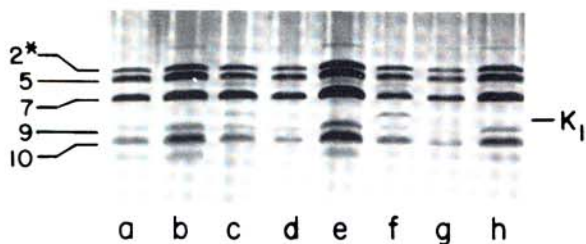

Fig. 1. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoregrams of cultivar Neepawa gluten proteins (origin at top): **a,** flour control, extracted with SDS + 2-mercaptoethanol; **b,** gluten control, extracted with SDS + 2-mercaptoethanol; **c,** acetic acid-soluble protein; **d,** urea-soluble protein; **e,** cetyltrimethylammonium bromide (CTMAB)-soluble protein (loaded at 2× the amount of protein in c and d); **f,** acetic acid/urea (AU)-soluble protein; **g,** urea/CTMAB (UC)-soluble protein; and **h,** acetic acid/urea/CTMAB (AUC)-soluble protein.

CTMAB addition increased the amount of protein extractable from wheat flour, but they noted a decrease in the relative amount of protein in the HMW fraction at the same time. They interpreted this effect as arising from a release of complexed gliadin from the HMW glutenin fraction. Results in Figure 1 suggest that inclusion of CTMAB as part of the AUC solvent interferes with extraction of the K1 protein by A or AU solvents (compare lanes c, f, and h). This suggestion is consistent with the report of Sullivan (1952), that CTMAB causes denaturation and precipitation of some wheat proteins. Since the samples c–h (Fig. 1) were treated with the same SDS/2-mercaptoethanol solvent for SDS-PAGE with which total proteins were extracted from flour and gluten for the "control" analyses, the absence of the K1 protein in the latter extracts was also interesting. At present, we can only speculate that SDS also interferes with the extraction of the K1 protein by modifying its ionic (hence hydrophilic) properties. This speculation is consistent with the results of Meredith and Wren (1966) and Sullivan (1952). The experience of one of us with ribosomal proteins showed (Chatterjee et al 1973) that when proteins are prepared for SDS-PAGE analysis, it is not mandatory that SDS must be used for extraction.

High Molecular Weight Proteins in Other Wheat Varieties

Figure 2 shows the SDS-PAGE of the proteins extracted by AU solvent from flour of Canadian wheat varieties Neepawa, Roblin, Marquis, Katepwa, Fielder, and HY320 (lanes a–f). The patterns of the corresponding standard extracts with SDS and 2-mercaptoethanol for SDS-PAGE are shown for comparison (lanes g–l). Comparison of lanes a–f showed a band comparable in mobility to that of the new HMW protein (K1) of Neepawa present in all. A pair of bands (K2 and K3) located between the HMW subunits 5 and 7 in the pattern of Neepawa also appears in the patterns of Marquis, Katepwa, Fielder, and HY320 (c–f). One of these two bands (K2) was absent in the analogous standard SDS-PAGE patterns. Another relatively strong band (K4) with a lower mobility than subunit 5 was observed in the extract of HY320 and is present as a faint band in the corresponding SDS-PAGE pattern of the flour.

The K proteins are distinct from the E glutenin bands of Gupta and Shepherd (1987) and the oligomeric bands of HMW glutenin subunits of Lawrence and Payne (1983) since these proteins have slower mobilities than the usual HMW glutenin subunits, and apparently their extraction is not affected by use of SDS.

We believe that the proteins described are unique natural proteins that are not produced by either degradation or aggregation of other proteins for the following reasons: 1) their extraction was achieved in total absence of commonly used denaturing and degrading substances, namely, SDS and CTMAB; 2) acetic acid and urea at concentrations used belong to a group of "good" solvents that produced no degradation when used in the extraction of ribosomal proteins (Chatterjee et al 1973, Kazemie 1975); and 3) we would have observed a reciprocal shift in the intensity of bands of some larger or smaller HMW glutenin subunits if they were degraded or aggregated into K proteins. Furthermore, a

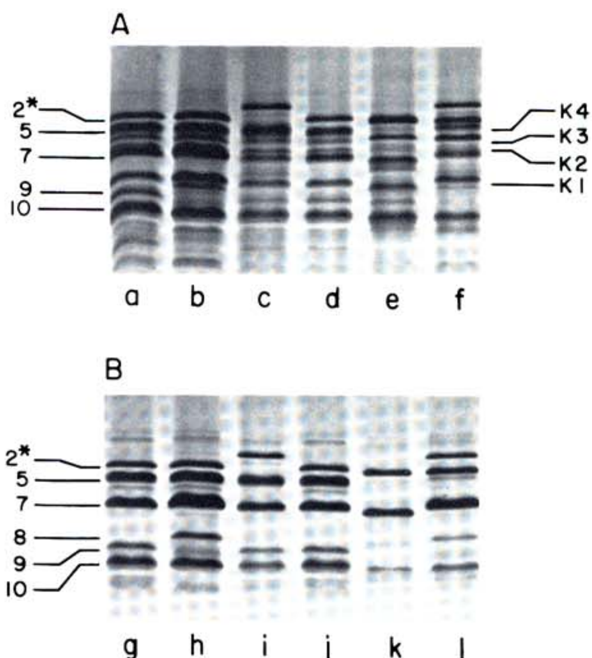

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams of acetic acid/urea-soluble (a–f) and total flour (g–l) proteins from flour of Canadian wheat varieties: **a** and **g,** Neepawa; **b** and **h,** Roblin; **c** and **i,** Marquis; **d** and **j,** Katepwa; **e** and **k,** Fielder; and **f** and **l,** HY320. The amount of protein loaded for each sample (a–l) was 2× the amount used in Fig. 1.

24-hr extraction of Neepawa did not change the relative proportion of K proteins to the HMW glutenin subunits (data not shown). When the residue of the AU extract was analyzed for HMW glutenin subunits by SDS-PAGE parallel with the proteins in the AU extract, the K proteins were present only in the latter case, indicating that these proteins are not produced during electrophoresis (results not shown). Additions of different concentrations of EDTA (ethylenediaminetetraacetic acid) and urea to the sample solution for electrophoresis also caused no change in the pattern of the proteins.

In summary, a unique group of HMW proteins is described herein that is extractable together with glutenin by acetic acid and by AU but not by AUC (AU + CTMAB). This differential pattern of extraction suggests that they are not linked covalently (i.e., by disulfide bonds) to the HMW glutenin subunits observed by standard SDS-PAGE analysis. The interaction could involve charged groups that could be blocked by detergents such as CTMAB and SDS, leading to denaturation during the process of extraction. Further work on characterization and functionality of the proteins reported in this paper is in progress.

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