NOTE

Presence of K Proteins in Developing Wheat Grain[1]

M. KAZEMIE[2] and W. BUSHUK

Previously we reported the identification of a new group of high molecular weight (HMW) proteins in some Canadian wheat varieties and referred to them as K proteins (Kazemie and Bushuk 1990). They were extractable with a solvent comprising acetic acid and urea but not with solvents that contained detergents. In this note, their presence in developing grain is reported in order to confirm their genuine nature.

MATERIALS AND METHODS

Wheat Sample

Neepawa, a cultivar of Canadian hard red spring bread wheat, was grown in a glass house. The heads were tagged on the day of anthesis and harvested at different days postanthesis (DPA) up to the stage of desiccation. The grains of the lower two thirds

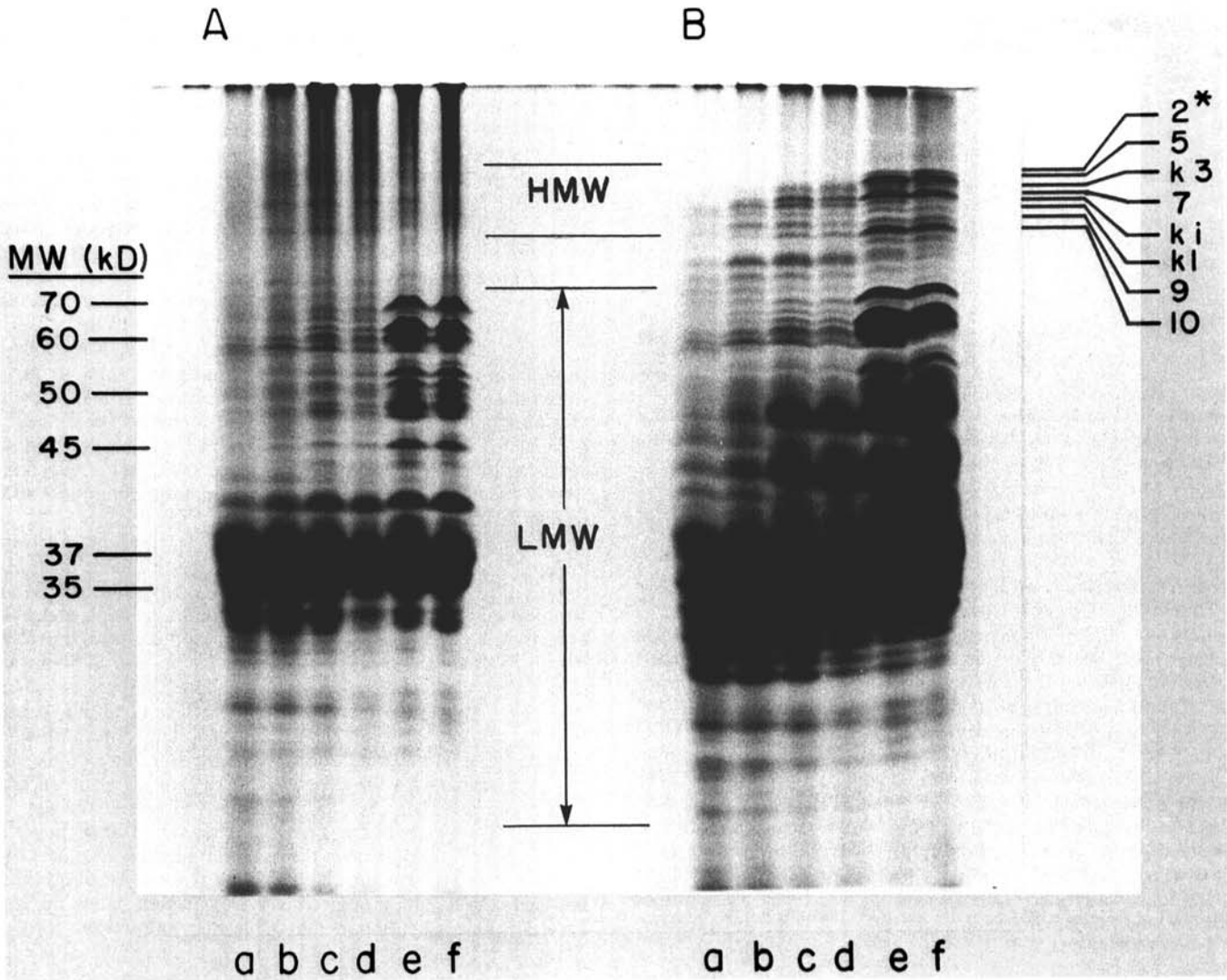

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns (origin at top) under nonreducing (**A**) and reducing (**B**) conditions of storage proteins from developing grain at various days postanthesis: a = 12, b = 15, c = 20, d = 25, e = 32, f = 40. Molecular weight (MW) scale was determined under reducing conditions using commercial proteins of known MW as markers. The same amount of protein was loaded for each lane. H = high, L = low.

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[2]Permanent address: 419-555 St. Mary Ave., Winnipeg, MB, Canada R3C 3X4.

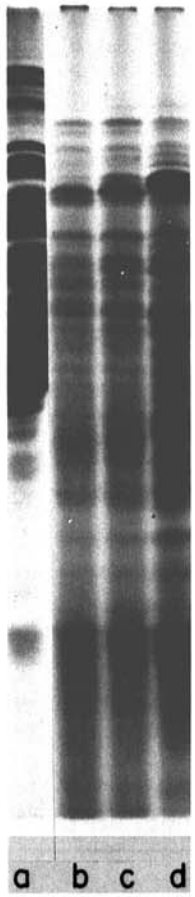

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of low salt-soluble proteins of developing grain. a = storage proteins of mature grain (control); b, c, and d = soluble proteins at 12, 25, and 40 days postanthesis, respectively.

of the heads were collected, frozen in liquid nitrogen, and kept frozen at −70°C until use.

Protein Extraction from Developing Seeds

The frozen grains were cut with a sharp blade to remove approximately one third of the kernel containing the embryo and ground in a mortar under liquid nitrogen. The powder was suspended in five volumes of a low-salt (low ionic strength) buffer (Kazemie 1971) containing 250 m*M* sucrose, 50 m*M* KCl, and 20 m*M* tris-HCl (pH 6.5). The suspension was centrifuged at 10,000 × *g* and 2°C for 10 min. The supernatant was dialyzed against double-distilled water and freeze-dried to yield soluble proteins. The residue was washed twice by dispersing in the same volume of the low-salt buffer and recentrifuging. Subsequently, the washed residue was extracted with three volumes of a solution comprising 0.1*M* acetic acid and 6*M* urea for 2 hr at room temperature as previously described (Kazemie and Bushuk 1990). The proteins extracted by this procedure are referred to as "storage" proteins.

Electrophoresis

Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was described elsewhere (Ng and Bushuk 1987, Kazemie and Bushuk 1990). Molecular weights were estimated by the procedure of Ng and Bushuk (1987). The HMW glutenin subunits were numbered according to Payne and Lawrence (1983). Protein content was determined by the Kjeldahl procedure.

RESULTS AND DISCUSSION

Protein Composition of Developing Grain

Figure 1 shows the SDS-PAGE patterns of storage proteins at different stages of grain development under nonreducing (A) and reducing (B) conditions.

The very first HMW proteins, observed under reducing conditions as early as 12 DPA and in order of decreasing mobility, were the HMW glutenin subunits 10 and 9 and the K proteins K1 and Ki (i = intermediate) (Fig. 1B, lane a). Then, at 15 DPA, HMW glutenin subunit 7 and K3 were observed (Fig. 1B, lane b). The pattern remained unchanged up to and including 25 DPA. At 32 DPA, the HMW glutenin subunits with the lowest mobilities (i.e., subunits 5 and 2*) were observed for the first time.

The SDS-PAGE patterns under reducing conditions of the soluble proteins at the same stages of development did not contain any bands with mobilities equal to those of the K proteins or the HMW glutenin subunits of the storage proteins (Fig. 2).

Low molecular weight (LMW) gliadins (33–40 kDa) were present as strong bands at 12 DPA (Fig. 1A and B, lanes a and b). At 20 DPA, LMW glutenin subunits with molecular weights of 40–50 kDa were observed as intense bands (compare Fig. 1A and B, lanes c and d). HMW gliadins (50–70 kDa) increased in intensity at 32 DPA and thereafter (compare lanes e and f in Fig. 1 A and B). It is not surprising that the young grain synthesizes essentially the LMW storage proteins first, otherwise the storage capacity of its cytoplasm would be soon overloaded. It is noteworthy that the storage proteins in developing seeds acquire the ability to form gluten mass around 30 DPA, as it could be observed by stirring with a glass rod the seeds homogenates in the low salt buffer (results not shown). However, more research is required before it can be stated with certainty whether this ability depends on the presence of a complete set of glutenin subunits or on the quantity of the storage proteins as a whole.

The presence of K1, Ki, and K3 proteins in developing wheat grain, but only K1 and K3 in the extract of flour of the same wheat cultivar (Kazemie and Bushuk 1990), leads to the following speculation about their physiological significance. A possible interpretation could be that they belong to the same group of proteins as do the HMW glutenin subunits but perhaps in some way are modified so that they cannot form interpolypeptide disulfide linkages (Kazemie and Bushuk 1990). Unlike K1 and K3, Ki diminished as maturity was reached. Whether its decline is related to the appearance of HMW glutenin subunits 5 and 2 remains to be investigated.

Qualitative and quantitative changes in total or individual groups of storage proteins during grain development have been reported by many researchers (for reviews, see Lasztity 1984 and Wrigley and Bietz 1988). However, the K proteins and some aspects of change in composition of the other storage proteins described here have not been reported. In the case of K proteins (Kazemie and Bushuk 1990), the choice of solvent is of considerable importance in relation to the results obtained. One possible reason why the phasic change in composition of wheat storage proteins has not been observed before may be the one-step procedures used for extraction of the storage proteins from developing grain (for reviews, see Lasztity 1984 and Wrigley and Bietz 1988). As shown in Figure 2, the SDS-PAGE bands of the soluble proteins overlap those of the gliadins and the LMW glutenin subunits. Accordingly, it is not possible to discriminate between soluble and storage (insoluble) protein bands by SDS-PAGE of an extract obtained by a one-step extraction.

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