NOTE

Identification of Some Wheat Proteins Separated by a Two-Step Acid Polyacrylamide Gel Electrophoresis and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis Technique

G. BRANLARD,[1] D. KHELIFI,[2] and G. LOOKHART[3]

The purpose of this study was to identify some proteins revealed by the recent two-step electrophoresis technique Khelifi and Branlard (1991) used to analyze the high molecular weight (HMW) and low molecular weight (LMW) subunits of glutenins.

Several electrophoretic techniques have been developed for analyzing the diversity of the LMW subunits of glutenins. The first technique used the two-step one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method of Singh and Shepherd (1988) and Gupta et al (1989). The seed proteins, extracted by a Tris-HCl buffer containing SDS, were electrophoresed on an SDS-PAGE gel. The smaller extracted proteins moved more quickly into the gel and were therefore separated from the aggregated proteins, which moved more slowly and remained in the first millimeters of the resolving gel, just under the stacking gel. After reduction, these aggregated proteins were subjected to a gradient SDS-PAGE gel to separate the HMW and LMW glutenin subunits and some nonstorage proteins.

The second technique was that described by Khelifi and Branlard (1991), in which nearly all storage proteins, together with other proteins, were extracted by 2-chloroethanol. First, the gliadins, albumins, and globulins were separated from the aggregated proteins using an acid-PAGE procedure. Then the aggregated proteins, which remained in the first millimeter of the acid gel, were extracted, reduced with β-mercaptoethanol, and subjected to SDS-PAGE to separate the HMW and LMW subunits of glutenin. Several recent reports give a simplification of these two-step procedures in analyzing HMW and LMW subunits of glutenin after one-dimensional SDS-PAGE (Gupta and MacRitchie 1991, Singh et al 1991, Zhen and Mares 1992).

When the same variety was compared using both two-step electrophoresis techniques, some differences were found in the patterns of the D-zone proteins (Khelifi and Branlard 1991). The acid-PAGE–SDS-PAGE technique showed heavily stained bands of M_r 50–67 kDa that were absent in the gel of the two-step one-dimensional SDS-PAGE of Singh and Shepherd (1988) and Gupta et al (1989). These three to five unknown bands, depending on the variety, seem to have a diverse mobility from one genotype to another. Therefore, some experiments were necessary to identify these D-zone proteins.

RESULTS AND DISCUSSION

The proteins extracted by 2-chloroethanol from individual half grains of Chinese Spring nullisomic (N)–tetrasomic (T) lines N1AT1B, N1AT1D, N1BT1A, N1BT1D, and N1DT1B were subjected to the two-step acid-PAGE–SDS PAGE separation. The corresponding patterns (Fig. 1) revealed that genes that code for the D-zone proteins were located on the group 1 chromosomes. Gupta and Shepherd (1987) found nonprolamin proteins in the D zone that were located on chromosome arms 4DL, 5BL, and 5AL. These nonprolamin proteins seem to be absent in the D-zone of chloroethanol extracts analyzed by the acid-PAGE–SDS-PAGE procedure. Figure 1 demonstrates that the genetic location is on the 1D chromosome for the two fastest bands and on the 1B chromosome for the other bands of Chinese Spring.

After SDS-PAGE of total reduced wheat proteins, Kasarda et al (1988) reported that the proteins with a mobility between those of the HMW and LMW subunits of glutenins were considered to be ω-gliadins. Consequently, the comparison of our D-zone proteins with the ω-gliadins seemed logical. The 2-chloroethanol extracted proteins from Chinese Spring were separated by acid-PAGE using a previously published method (Branlard et al 1990). The left and right edges of the gliadin acid gel were excised and rapidly stained with Coomassie blue to identify the mobility of the ω-gliadins. Five pieces of the unstained gel, corresponding to the five most heavily stained ω-gliadin bands of Chinese Spring identified on the edges of the stained gel, were cut. The proteins from each piece of gel were eluted with 70% ethanol overnight and then desicated with vacuum at ambient temperature. The collected proteins were equilibrated 10 min in a Tris-SDS pH 6.8 buffer and separated by SDS-PAGE. The results are shown in Figure 2. The mobility of the most heavily stained ω-gliadins correspond to those of the D-zone proteins. The 1DS ω-gliadins corresponding to the *Gli-D1* locus, with two bands on the acid gel for Chinese Spring, show a mobility identical to those of the bands previously located on 1D in our D-zone (Fig. 2, Lanes 4 and 5). Because Chinese Spring does not possess ω-gliadins corresponding to *Gli-A1* (Metakovsky

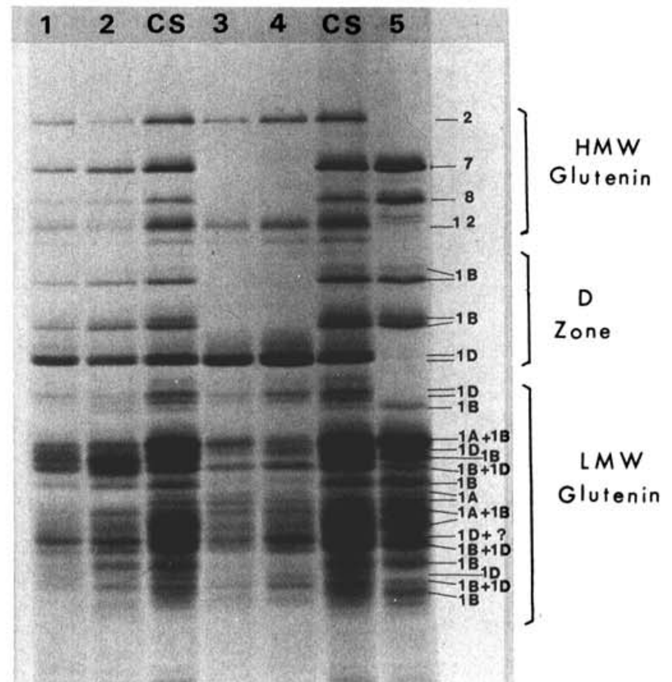

Fig. 1. Two-step acid-PAGE–SDS-PAGE patterns of the 2-chloroethanol seed proteins extracted from the Chinese Spring (CS) nullisomic (N)–tetrasomic (T) lines. Lane 1, N1AT1D; Lane 2, N1AT1B; Lane 3, N1BT1A; Lane 4, N1BT1D; Lane 5, N1DT1B. HMW = high molecular weight. LMW = low molecular weight.

[1]INRA Station d'Amélioration des Plantes, 63039 Clermont-Ferrand, France.
[2]Institut de Biologie, Université de Constantine, Constantine, Algérie.
[3]U.S. Department of Agriculture, Agricultural Research Service, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. American Association of Cereal Chemists, Inc., 1992.

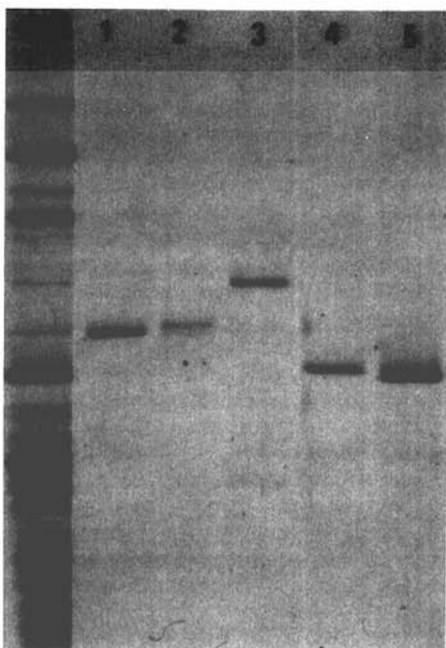

Fig. 2. Comparison of the mobility patterns of the ω-gliadins eluted from the acid gel with the mobility patterns of the D-zone proteins of Chinese Spring (CS) after two-step acid-PAGE–SDS-PAGE technique. Lanes 1–3: ω-gliadins coded by *Gli-B1* locus. Lanes 4 and 5: ω-gliadins coded by *Gli-D1* locus.

et al, 1984), the three other D-zone bands correspond to the 1BS ω-gliadins (Fig. 2, Lanes 1–3).

Using the two-step acid-PAGE–SDS-PAGE technique, we found that the D-zone proteins correspond to ω-gliadins. These ω-gliadins remained aggregated to the HMW and LMW glutenins upon initial extraction with 2-chloroethanol. Bietz et al (1975a,b) showed that glutenins do strongly associate noncovalently with gliadins. Because they were aggregated to the glutenins, some of these ω-gliadins may have functional properties different from those of the other gliadins.

ACKNOWLEDGMENTS

Y. Cauderon (INRA, Versailles, France) is gratefully acknowledged for providing the nullitetrasomic lines of Chinese Spring created by E. Sears (USDA-ARS, Columbia, MO).

LITERATURE CITED

BIETZ, J. A., and WALL, J. S. 1975a. The effect of various extractants on the subunit composition and associations of wheat glutenin. Cereal Chem. 52:145.

BIETZ, J. A., SHEPHERD, K. W., and WALL, J. S. 1975b. Single-kernel analysis of glutenin: Use in wheat genetics and breeding. Cereal Chem. 52:513.

BRANLARD, G., PICARD, B., and COURVOISIER, C. 1990. Electrophoresis of gliadins in long acrylamide gels: Methods and nomenclature. Electrophoresis 11:310.

GUPTA, R. B., and SHEPHERD, K. W. 1987. Genetic control of LMW glutenin subunits in bread wheat and association with physical dough properties. In: Proc. Int. Workshop on Gluten Proteins, 3rd. R. Lasztity and F. Bekes, eds. World Scientific, Singapore.

GUPTA, R. B., SINGH, N. K., and SHEPHERD, K. W. 1989. The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theor. Appl. Genet. 77:57.

GUPTA, R. B., and MacRITCHIE, F. 1991. A rapid one-step one dimensional SDS-PAGE procedure for analysis of subunit composition of glutenin in wheat. J. Cereal Sci. 14:105.

KASARDA, D. D., TAO, H. P., EVANS, P. K., ADALSTEINS, A. E., and YUEN, S. W. 1988. Sequencing of protein from a single spot of a 2-D gel pattern: N-terminal sequence of a major wheat LMW-glutenin subunit. J. Exp. Bot. 39:899.

KHELIFI, D., and BRANLARD, G. 1991. A new two step electrophoresis method for analyzing gliadin polypeptides and high and low molecular weight subunits of glutenin of wheat. J. Cereal Sci. 13:41.

METAKOVSKY, E. V., NOVOSELSKAYA, A. Y., KOPUS, M. M., SOBKO, T. A., and SOZINOV, A. A. 1984. Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis. Theor. Appl. Genet. 67:559.

SINGH, N. K., and SHEPHERD, K. W. 1988. Linkage mapping of genes controlling endosperm storage proteins in wheat. I. Genes on the short arms of group 1 chromosomes. Theor. App. Genet. 75:628.

SINGH, N. K., SHEPHERD, K. W., and CORNISH, G. B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J. Cereal Sci. 14:203.

ZHEN, Z., and MARES, D. 1992. A simple extraction and one-step SDS-PAGE system for separating HMW and LMW glutenin subunits of wheat and high molecular weight proteins of rye. J. Cereal Sci. 15:63.

[Received May 24, 1991. Accepted July 18, 1992.]