NOTE

Detectability of Different Classes of Gliadins by Dye Binding and by Trichloroacetic Acid Precipitability[1]

K. KHAN, Department of Cereal Chemistry and Technology, North Dakota State University, Fargo 58105

ABSTRACT

Different classes of gliadin proteins of hard red spring, hard red winter, and durum wheats were detected on polyacrylamide gels after treatment of the gel with Coomassie brilliant blue or 12% (w/v) trichloroacetic acid (TCA). The TCA detection method, though less sensitive than the Coomassie brilliant blue protein stain, was especially useful in locating two gliadin component bands, 42 and 45, associated with durum wheat quality. Gliadin components can be detected by the TCA method just 10–15 min immediately after electrophoresis. Extraction and centrifugation time of gliadin for electrophoresis was only 10 min.

Cereal Chem. 61(4): 378-380

Damidaux et al (1978, 1980) showed that two gliadin components, designated as bands 42 and 45, were associated with gluten strength of durum wheats. Cultivars (varieties) that contained band 42 but lacked band 45 possessed weaker gluten than cultivars that contained band 45 but lacked band 42. Similar relationships between bands 42 and 45 and gluten strength were found for Canadian (Kosmolak et al 1980) and Australian (DuCross et al 1982) durum wheats. Quick et al (1979a, 1979b) reported on the advantages in pasta making of North Dakota durum varieties such as Edmore and Vic that possess strong gluten. Polyacrylamide gel electrophoresis (PAGE) of the gliadins of Edmore and Vic (Khan 1982) showed the presence of band 45 in these two durum varieties.

The lack of rapid detection methods for proteins on polyacrylamide gels has been one of the major disadvantages of the PAGE procedure. This article deals with the rapid extraction of gliadin and the detection of different classes of gliadins, especially bands 42 and 45, which are gliadin components associated with durum wheat quality. These rapid procedures can be of use especially if PAGE is employed in early-generation screening in durum wheat quality research.

MATERIALS AND METHODS

Wheat Samples

The wheat varieties used in this study were obtained from the Seeds Stock Project, Department of Agronomy, North Dakota State University, Fargo.

Extraction of Gliadin

Ground grain (meal) (Bushuk and Zillman 1978) was extracted as follows: 1.0 ml of 70% aqueous ethanol was added to 0.5 g of meal in a 15-ml Corex test tube. The preparation was then shaken on a vortex mixer for 5 sec and left standing at room temperature for 5 min. The sample was then centrifuged for 5 min at 20,000 × g at 20° C. The supernatant was used for PAGE (Khan et al 1983).

A second preparation was placed in a Burrel Wrist-Action Shaker, model DD (Burrel Corp., Pittsburg, PA), and shaken continuously for 5 min. The sample was then centrifuged and the supernatant retained for PAGE as above. A third preparation was vortexed continuously for 5 min, and a fourth sample was placed in a water bath at 50° C for 5 min. Both preparations were centrifuged and their supernatants retained as above for PAGE.

Single kernels also were ground in a mortar with a pestle and the meal transferred to a test tube. Then 0.10 ml of 70% aqueous ethanol was added to the meal and extracted for 5 min either by shaking (Burrel shaker) or by standing, as above.

Gliadin was also extracted according to the conventional 1-hr method according to Khan et al (1983).

All extractions were done at least two times and the gliadins subjected to PAGE to check for the reproducibility of the extraction procedures.

PAGE

The PAGE procedure used in this study was that of Bushuk and Zillman (1978), as modified by Khan et al (1983). The E-C 470 and 490 vertical gel cell (E-C Corporation, St. Petersburg, FL) were used in this study. For these studies, gels 3 mm thick were used instead of gels 6 mm thick, as previously described (Khan et al 1983).

Detection of Gliadin Components with Trichloroacetic Acid (TCA) and Coomassie Brilliant Blue (CBB)

After PAGE, the gel was placed in a transparent tray of Pyrex or similar material, and enough 12% TCA was added to cover the gel. The glass tray was then placed on a shaker and shaken gently to keep the gel suspended in the TCA solution. If a shaker is not available, the tray can be shaken by hand every 3–4 min. After approximately 10–15 min of shaking, the tray was then placed against a black background. The gliadin components are visible as white bands against the black background. The bands can be seen more clearly if the gel is illuminated from the sides with fluorescent light either in the tray or on a glass plate against the black background. The gels can be stored in the 12% TCA solution.

The TCA-treated gels were stained with CBB according to Bushuk and Zillman (1978).

Photography of Polyacrylamide Gels

Gels treated with TCA were placed on a piece of transparent glass against a black background such as a black piece of cloth or cardboard. The gel was then illuminated from the right and left sides with fluorescent light. A 135-mm camera mounted on a stand was placed directly above the gel, but the camera cast a shadow on the gel. The shadow was eliminated by allowing only the camera lens to protrude slightly through a hole cut in the middle of a black piece of cardboard (35 × 60 cm). The black cardboard was kept in place above the gel while photographs were taken on Kodak technical pan film 2415 (ESTAR-AH Base) at an ASA value of 100. Gels stained with CBB were photographed as described by Khan et al (1983).

RESULTS AND DISCUSSION

Trichloroacetic acid is commonly used as a precipitating or denaturing agent for proteins. This property of TCA is also utilized in many PAGE procedures in which TCA is used to "fix" or precipitate proteins on polyacrylamide gels after electrophoresis is complete. After electrophoresis, the gel is either treated with TCA before the protein staining solution is added to it, or the TCA and the staining solution are mixed together and then added to the gel.

[1]Published with the approval of the director of the Agricultural Experiment Station, North Dakota State University, Fargo 58105, as Journal Series No. 1303.

The main reason for fixing or precipitating the proteins on the gel is to minimize any possible lateral diffusion of the protein components, thereby retaining resolution on the gel.

During investigations of wheat proteins by PAGE, several gliadin components of both tetraploid and hexaploid wheats could be detected on gels by TCA treatment of the gels in the absence of protein staining solution. After TCA treatment, the protein components appear as white bands against a black background. Only 10–15 min is required to detect the protein bands on gels 3 or 6 mm thick. The TCA detection method was especially useful in locating the two gliadin components of durum wheats, bands 42 and 45, which are associated with gluten strength (Damidaux et al 1978, 1980; Kosmolak et al 1980; DuCross et al 1982). A TCA-treated gel is shown in Fig. 1. Bands 42 and 45 (Fig. 1) are quite distinctly separated and appear as white bands. (The bands are clearer on the actual gel than they appear in the photographs of Figs. 1 and 4.) Values 42 and 45, relative mobility values, are obtained with reference to band 51 (Fig. 1), according to Bushuk and Zillman (1978) and Damidaux et al (1978). Of the durum varieties examined by PAGE (Fig. 1), only Coulter, Edmore, Lloyd, Vic, and Yuma show band 45. All other varieties of Fig. 1 show band 42. Lloyd is the first semidwarf durum variety released in North Dakota (Cantrell and Dick 1983) that possesses strong gluten characteristics.

Figure 2 shows the same gel of Fig. 1 stained with the protein stain CBB. Whereas the TCA treatment detected bands mostly in the midmobility region of the gel (β and γ gliadins), the CBB stain is more sensitive in detecting bands in all regions of the gel.

Figure 3, A and B, compare the gliadin patterns of some hexaploid and tetrapolid wheats by the TCA detection and CBB staining methods, respectively. As mentioned before, the components in the midmobility region of the gel (Fig. 3A) are detectable on the TCA-treated gel. It is, however, interesting to note in Fig. 3A (TCA gel) that the components in the α region of the hexaploid wheats are extremely faint, whereas those of the tetraploid wheats are not detectable, even though many of those gliadin bands seem to be of equal staining intensity with components in the β and γ regions, as shown in the CBB stained gel. Perhaps structural differences between these two classes of gliadins may be responsible for this difference in TCA precipitability and dye-binding capacity of these proteins. Greene and Kasarda (1971) have shown that the α gliadins possess a large degree of hydrophobicity. The gliadin components in the ω region of the gel are also detectable on the TCA-treated gel. However, most of these components are faint mostly because of the smaller amounts of these components indicated by the less intensely stained bands on the CBB gel (Fig. 3B). The TCA treatment is, therefore, primarily limited to detection of those gliadin proteins in the midmobility region of the gel. However, the TCA method is much faster in detecting gliadin bands in the β and γ regions than the CBB stain.

Figure 4 shows the TCA method of detection on polyacrylamide gel of gliadin extracted by various rapid methods compared to the conventional 1-hr method of extraction. Two durum wheat varieties, representing the weaker (Rugby) and stronger (Vic) gluten types, were chosen for the rapid-extraction studies. The PAGE patterns of all extracts of Rugby (patterns 1–7 and 15–18), containing band 42, are all qualitatively identical to the conventional 1-hr extract. Similarly, PAGE patterns of all extracts of Vic (patterns 8–14 and 19–22), containing band 45, are all qualitatively identical except pattern 11, which was obtained from a sample that was not shaken during extraction.

Figure 5 shows the same gel as in Fig. 4 stained with CBB; the CBB-stained components of both Rugby and Vic are qualitatively identical. Pattern 11, as mentioned earlier, shows faint bands with the CBB stain also. Patterns 6 and 7 (Rugby) and 13 and 14 (Vic)

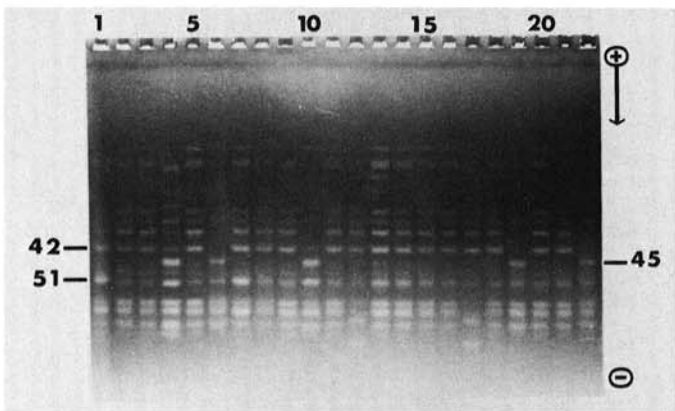

Fig. 1. Polyacrylamide gel electrophoresis patterns obtained by trichloroacetic acid treatment of the gliadins of the following durum wheat varieties: 1, Botno; 2, Calvin; 3, Cando; 4, Coulter; 5, Crosby; 6, Edmore; 7, Lakota; 8, Langdon; 9, Leeds; 10, Lloyd; 11, Mindum; 12, Ramsey; 13, Rolette; 14, Rugby; 15, Sentry; 16, Stewart; 17, Towner; 18, Vernun; 19, Vic; 20, Ward; 21, Wells; and 22, Yuma. Samples were extracted from meal according to the conventional 1-hr procedure.

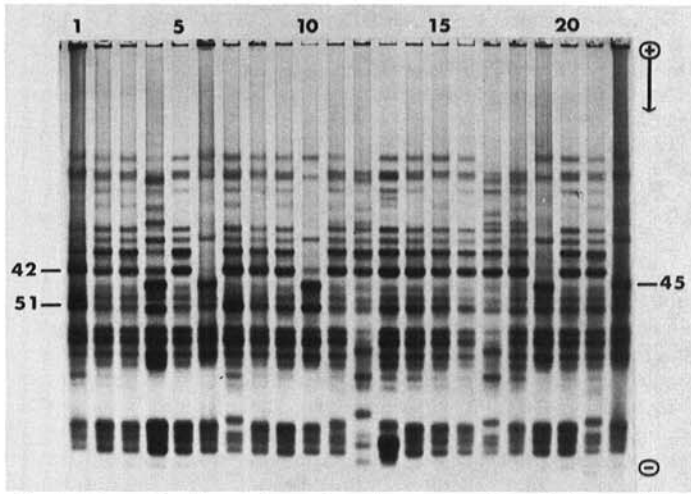

Fig. 2. Polyacrylamide gel electrophoresis patterns of the gliadins obtained after the trichloroacetic acid-treated gel of Fig. 1 was stained with Coomassie brilliant blue. The varieties 1–22 are the same as in Fig. 1.

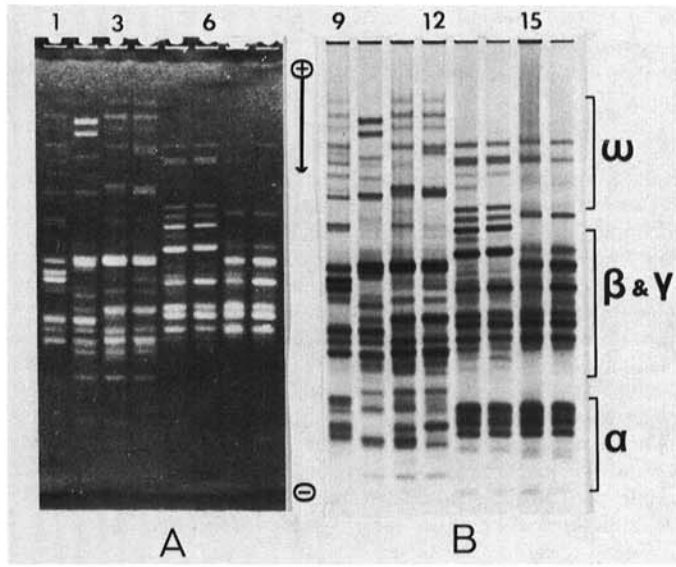

Fig. 3. A, Polyacrylamide gel electrophoresis patterns obtained by trichloroacetic acid treatment of gliadins of hexaploid and tetraploid wheat: 1,2, the hard red spring wheats Len and Olaf; 3,4, the hard red winter wheats Roughrider and Winoka; and 5–8, the durum (tetraploid) wheats Cando, Langdon, Edmore, and Vic. Samples were extracted from meal according to the 1-hr procedure. **B,** Polyacrylamide gel electrophoresis patterns of gliadins obtained after the trichloroacetic acid-treated gel of Fig. 3A was stained with Coomassie brilliant blue. Patterns 9–16 are from the same varieties as for 1–8 of Fig. 3A.

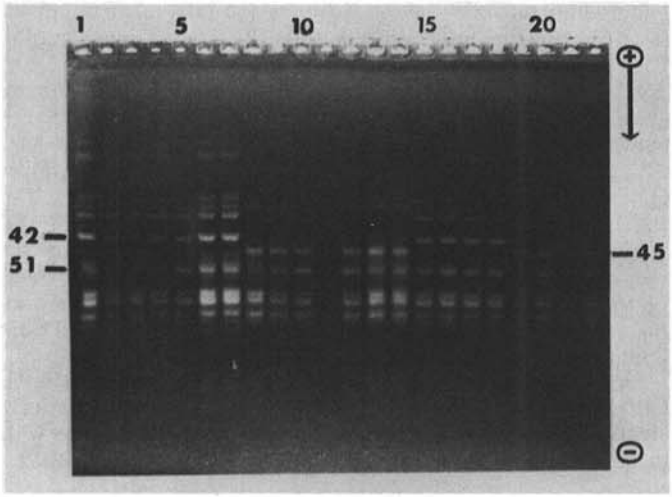

Fig. 4. Comparison of polyacrylamide gel electrophoresis patterns on trichloroacetic acid-treated gel of gliadin extracts of the durum varieties Rugby (patterns 1–7 and 15–18) and Vic (patterns 8–14 and 19–22) obtained by various extraction procedures: 1,8, 1-hr extract of whole meal; 2,9, 5-sec vortexing followed by 5 min extraction without shaking; 3,10, extraction by shaking for 5 min on Wrist-Action shaker; 4,11, single-kernel extraction as in 2 and 9; 5,12, single-kernel extraction as in 3 and 10; 6,13, meal extracted by vortexing continuously for 5 min; 7,14, meal extracted without shaking at 50° C in a water bath. Patterns 15–18 (same sequence of extraction as for 2–5) and 19–22 (same sequence of extraction as for 9–12) were obtained from extractions centrifuged on a bench-top centrifuge.

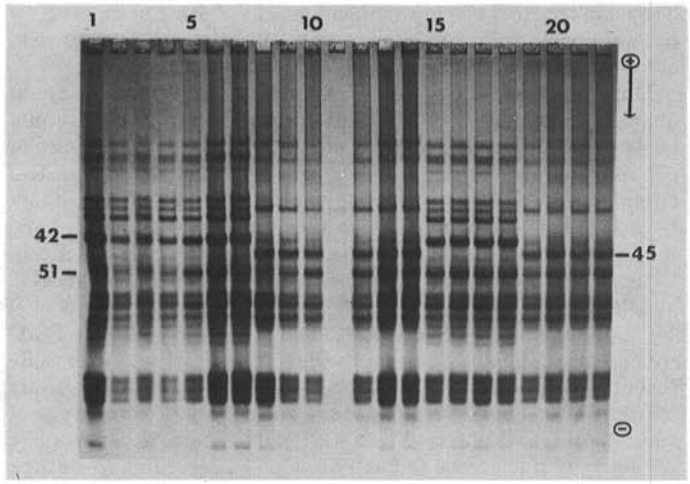

Fig. 5. Comparison of the polyacrylamide gel electrophoresis patterns obtained after the trichloroacetic acid-treated gel of Fig. 4 was stained with Coomassie brilliant blue. The order of extractions are the same as in Fig. 4.

obtained from gliadin extracted for 5 min by continuous vortexing and at 50° C in a water bath, respectively, are both qualitatively and quantitatively similar to the conventional 1-hr extract (patterns 1 and 8, respectively). Patterns 15–23 were obtained from gliadin that was extracted either by shaking (Burrel shaker) or by being left to stand at room temperature for 5 min. These samples, however, were centrifuged on a bench-top centrifuge (International Equipment Co., Boston, MA, model HT) at 3,000 × *g*. These patterns are qualitatively identical to the other patterns, which were obtained from centrifugation at 20,000 × *g* on a Sorvall RC-5 Superspeed refrigerated centrifuge (DuPont Instruments, Newtown, CT). Therefore, for extraction of gliadin components, a procedure that involves shaking the meal for 5 min should give a gliadin preparation suitable for PAGE and for subsequent detection of the protein components of interest by TCA treatment and/or by CBB stain.

Tkachuk and Metlish (1980) have recommended the Bio-Rad model 220 (now called the Protean) Dual Vertical Slab Cell (Bio-Rad Laboratory, Mississauga, Ontario, Canada) for gliadin PAGE because 40 samples can be electrophoresed with this apparatus at the same time in 40–80 min. However, it requires at least 1–2 hr (but usually overnight) to detect the protein components with CBB stain (Tkachuk and Metlish 1980). Because of its rapidity, the TCA detection procedure, coupled with multisample application with an apparatus such as the Bio-Rad 220 gel cell, can greatly accelerate the number of samples that can be analyzed by PAGE for detection of bands 42 and 45 in durum wheats. Bands 42 and 45 of experimental varieties can easily be located on polyacrylamide gels by use of reference varieties such as Rugby and Vic that have bands 42 and 45, respectively. Relative mobility values for all visible bands can also be calculated because the reference band 51 (Damidaux et al 1978) is clearly visible on TCA-treated gels.

CONCLUSION

A rapid technique utilizing a well-known protein precipitant, TCA, was used for the detection on polyacrylamide gels of gliadin components in the β and γ regions. The TCA detection method, though less sensitive than the protein stain CBB, is especially useful for locating bands 42 and 45, which are associated with gluten strength of durum wheats. The rapid TCA detection method, coupled with rapid extraction of gliadin and multisample application to the polyacrylamide gel, can have great potential as a screening technique for specific gliadin protein components if PAGE is utilized in durum wheat quality research.

ACKNOWLEDGMENTS

The part-time technical assistance of Samuel O'Tong is gratefully appreciated. Also, the financial assistance of the Minnesota Wheat Research and Promotion Council for part of this work is greatly appreciated.

LITERATURE CITED

BUSHUK, W., and ZILLMAN, R. R. 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Can. J. Plant Sci. 58:505.

CANTRELL, R. G., and DICK, J. W. 1983. Durum wheat improvement update. Macaroni J. 65:32.

DAMIDAUX, R., AUTRAN, J. C., GRIGNAC, P., and FEILLET, P. 1978. Mise en evidence de relations aplicables en selection entre l'electrophoregramme des gliadines et les proprietes viscoelastiques du gluten de *Triticum durum*. Desf. C. R. Acad. Su. Ser. D. 287:701.

DAMIDAUX, R., AUTRAN, J. C., GRIGNAC, P., and FEILLET, P. 1980. Gliadin electrophoregrams and measurements of gluten viscoelasticity in durum wheats. Cereal Foods World 25(12):754.

DuCROSS, D. L., WRIGLEY, C. W., and HARE, R. A. 1982. Prediction of durum-wheat quality from gliadin-protein composition. Aust. J. Agric. Res. 33:429.

GREENE, F. C., and KASARDA, D. D. 1971. Apola interaction of alpha-gliadin: Binding of 2-*p*-toluidinylnaphtalene-6-sulfonate. Cereal Chem. 48:601.

KHAN, K. 1982. Polyacrylamide gel electrophoresis of wheat gluten proteins. Bakers Dig. 56:14.

KHAN, K., McDONALD, C. E., and BANASIK, O. J. 1983. Polyacrylamide gel electrophoresis of gliadin proteins for wheat variety identification–Procedural modifications and observations. Cereal Chem. 60:178.

KOSMOLAK, F. G., DEXTER, J. E., MATSUO, R. R., LEISLE, D., and MARCHYLO, B. A. 1980. A relationship between durum wheat quality and gliadin electrophoregrams. Can. J. Plant Sci. 60:427.

QUICK, J. S., DONNELLY, B. J., and MILLER, J. D. 1979a. Edmore ... North Dakota's first strong gluten durum. N.D. Agric. Exp. Stn. Farm Res. 36:14.

QUICK, J. S., DONNELLY, B. J., and MILLER, J. D. 1979b. Vic ... High yielding, strong gluten durum. N.D. Agric. Exp. Stn. Farm Res. 37:7.

TKACHUK, R., and METLISH, V. J. 1980. Wheat cultivar identification by high voltage gel electrophoresis. Ann. Technol. Agric. 29:207.

[Received August 26, 1983. Revision received January 30, 1984. Accepted January 30, 1984]