

Resolution of High Molecular Weight Glutenin Subunits by a New SDS-PAGE System Incorporating a Neutral pH Buffer

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Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been used extensively by researchers, geneticists, and plant breeders to analyze wheat proteins since its introduction to cereal chemists by Bietz and Wall in 1972 (see, for example, almost any recent issue of *Cereal Chemistry*; Payne et al 1987). Despite this common usage, SDS-PAGE remains something of an art. It is, for example, difficult to resolve certain combinations of high molecular weight glutenin subunits (HMW-GS), such as 2, 2* and 17, 18 (see Payne et al 1984 for numbering system) with the usual pH 8.6 Tris-glycine system (Laemmli and Favre 1973). This is especially true for small format gels (mini-gels).

Precast gels are available from a number of companies. These are convenient to use and have the added advantage that users are not exposed to the toxic acrylamide monomer. Although we have not made a complete survey of the market, we have evaluated about a half-dozen different commercial precast gels based on the Tris-glycine buffer system, including those from Novex (San Diego, CA). We were unable to resolve 2, 2* or 17, 18 in any of these Tris-glycine gels, and most did not do well with the 2*, 5 and 9, 10 combinations as well. We did find that a recently introduced precast gel electrophoresis system based on neutral pH buffers rather than basic pH buffers, the Novex NuPAGE system, resolved all the different HMW-GS in six different cultivars. These cultivars had diverse types of HMW-GS that were chosen to test the system. We were highly impressed by the capability of the system to resolve the HMW-GS and other wheat endosperm proteins and felt that this system might be useful to other investigators. Consequently, we provide here an example of the results obtained for the NuPAGE system in comparison with a Tris-glycine mini-gel of about the same size that had been cast and run in our laboratory according to our usual methods, which are close to the those recommended by Laemmli and Favre (1973).

MATERIALS AND METHODS

Total protein extracts from flours (endosperm only) of five different cultivars (Yecora Rojo, Cheyenne, Scout 66, Red River 68, and Chinese Spring) and seeds of one cultivar (Atlas 66) were analyzed. Flour samples (5 mg) were extracted with 300 μ L of extraction buffer (see below). An Atlas 66 seed weighing 28 mg was crushed in a mortar and pestle and ground with 600 μ L of extraction buffer. We used the Novex Xcell II Mini-Cell electrophoresis apparatus with a NuPAGE gradient gel (4–12%) Bis-Tris [Bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane-HCl]

with crosslinker concentration range of 3.8–5% depending on the region of the gel. The gels do not contain SDS. We used the Novex MES [2-(N-morpholino) ethane sulfonic acid] running buffer with NuPAGE antioxidant added to the upper chamber. A preliminary comparison was made between the 4–12% gradient gels and the single concentration 10% gels and also between the MES buffer and the MOPS [3-(N-morpholino) propane sulfonic acid] buffer (all from Novex). Recommended Novex procedures and solutions were used, with the exception of the sample and reduction solutions. The *Novex NuPAGE Electrophoresis System Technical Guide* was also helpful. Although the combination of Novex sample buffer and reducing agent gave satisfactory results, we had a slight preference for our own sample buffer for flour and seed extractions and this was used here. The formulation was 2% SDS, 10% glycerol, 50 mM dithiothreitol, 62.5 mM Tris (hydroxymethyl) amino methane—HCl, pyronin Y as marker dye (0.006%), pH 8.5. We preferred the pH 8.5 sample buffer to the usual pH 6.8 buffer because it seemed more effective in producing complete reduction with no x-y dimers (Werner et al 1992) and had no obvious deleterious effects on the patterns. The Novex Mark 12 mixture of molecular weight standards was included on gels. Sample loading was 9 μ L per slot. Run time was 70 min at 150 V.

A single concentration, 12% (11.84% acrylamide; 0.16% bis-acrylamide) Tris-glycine gel (pH 8.6) was cast and used for comparison with the NuPAGE gel. Methods were those described in Bio-Rad (Hercules, CA) instruction literature with minor modifications (Werner et al 1992). The gel separation was performed in the Bio-Rad Mini-Protean II apparatus. Sample buffer, extractions, and loading were the same as for the NuPAGE gel. The analysis was performed for 30 min at 150 V and then for 45 min at 200 V.

Staining of both types of gel was according to the Coomassie Brilliant Blue G-250 method of Neuhoff et al (1988) without trichloroacetic acid fixation. Staining was usually performed overnight, but an extra day improved staining of low molecular weight proteins in the high percentage range of the 4–12% gel. Destaining was with water only for a few hours.

RESULTS AND DISCUSSION

Figure 1 illustrates the protein patterns we obtained for the extracts of the six test cultivars on the NuPAGE 4–12% gel. Figure 2 illustrates these same patterns for the 12% Tris-glycine gel.

The gel patterns have been divided into four regions of migration for purposes of discussion and to be in accord with common usage. Region A corresponds mainly to the HMW-GS, Region D corresponds to ω -gliadins and D glutenin subunits, and both Regions B and C correspond to gliadins and low molecular weight glutenin subunits (LMW-GS) with LMW-GS usually predominating in Region B and gliadins usually predominating in Region C.

The HMW-GS in the patterns of each cultivar had been assigned in earlier comparisons (not shown) with those of standard cultivars in which assignments had been made by P. I. Payne (*personal communication*). These assignments are (in order of band migration in Tris-glycine gels—slowest to fastest): Atlas 66,

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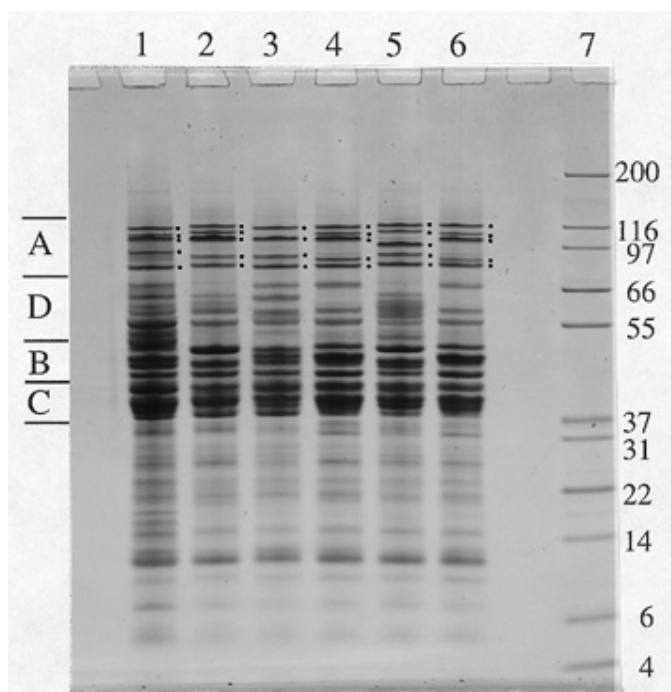


Fig. 1. SDS-PAGE of protein extracts from six different cultivars in a 4–12% gradient NuPAGE gel (MES buffer, pH 7.3). Lane 1, Atlas 66 (seed extract); Lane 2, Red River 68; Lane 3, Chinese Spring; Lane 4, Scout 66; Lane 5, Yecora Rojo; and Lane 6, Cheyenne. Molecular masses of standards shown in kilodaltons. Gel dimensions: 1 × 80 × 80 mm.

2, 2*, 13, 16, 12; Red River 68, 1, 5, 7, 8, 10; Chinese Spring, 2, 7, 8, 12; Scout 66, 2, 2*, 7, 9, 12; Yecora Rojo, 1, 5, 17, 18, 10; and Cheyenne; 2*, 5, 7, 9, 10. Dots alongside the bands in Fig. 2, Region A, correspond to the HMW-GS in the order given. Side-by-side dots indicate the overlapping of 2, 2* in Atlas 66 and Scout 66 and of 17, 18 in Yecora Rojo. Dots alongside the Region A bands of Fig. 1, the NuPAGE gel, are in the same order as usually found in Tris-glycine gels, except that 1, 5 are reversed in Red River 68 (becoming 5, 1) and 2*, 5 are reversed in Cheyenne (becoming 5, 2*). The reversals were assigned by comparing band intensities and mobilities.

All major HMW-GS of the cultivars compared were apparently resolved in the NuPAGE gel. Note the exceptional resolution of 17, 18 in Yecora Rojo (Fig. 1, Lane 5). Although we have sometimes resolved all the band combinations found in the cultivars analyzed in a single laboratory-cast Tris-glycine gel, this has been rare, we have never achieved this in the mini-gel format, and we have never achieved this in a gel with useable resolution ranging from approximately 250,000 down to approximately 4,000 in molecular weight as for the NuPAGE gel.

Novex claims that the NuPAGE gels can be stored for up to one year, whereas they put a 10-week expiration date on their precast Tris-glycine gels. This improved stability results presumably from the neutral pH of the NuPAGE gels in contrast to the more basic pH of Tris-glycine gels. Although we have not stored NuPAGE gels for more than a month or two, we have been favorably impressed by the reproducibility of results with different shipments of gels over a 10-month period.

Resolution of the LMW-GS was also excellent. Although we did not make a rigorous comparison, the single concentration 10% gels seemed nearly equivalent in resolution of HMW-GS and perhaps slightly better in the resolution of LMW-GS than the 4–12% gradient gels, but the single concentration gel did not have the exceptional molecular weight range of the gradient gel (data not

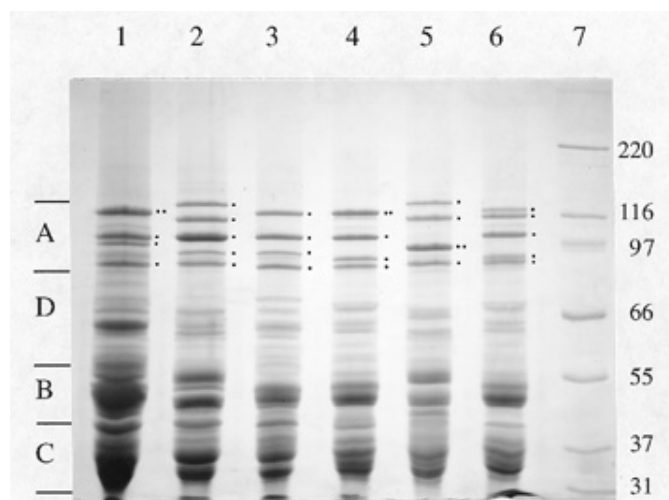


Fig. 2. Same protein extracts as for Fig. 1 analyzed on a custom-cast Tris-glycine gel (12%), pH 8.6. Lane 1, Atlas 66 (seed extract); Lane 2, Red River 68; Lane 3, Chinese Spring; Lane 4, Scout 66; Lane 5, Yecora Rojo; and Lane 6, Cheyenne. Molecular masses of standards shown in kilodaltons. Gel dimensions were 1×80×73 mm.

shown). Again, we did not make a rigorous comparison, but preliminary experiments indicated that results were somewhat better for wheat endosperm proteins when the MES buffer was used, as opposed to the MOPS buffer (data not shown). For most endosperm proteins, the small format gels were the equivalent (usually better) in resolution of larger format gels and we did not encounter any difficulties in viewing or photographing the patterns.

In conclusion, we think the excellent resolution of HMW-GS, good resolution in general, broad range, convenience, speed, absence of exposure to acrylamide monomer that occurs during gel casting, and long-term storability make the NuPAGE gels suitable for general use in laboratories where HMW-GS and other wheat endosperm proteins are analyzed by SDS-PAGE. They may be especially useful when analyses need to be made only occasionally, requiring that researchers and technicians re-prepare complete sets of stock solutions and regain expertise with high-resolution SDS-PAGE methods after long lay-off periods, or when large numbers of analyses must be made in a short time.

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