

Enhanced Electrophoretic Detection and Isolation of Friabilin, a Starch Granule Protein¹

To the Editor:

Endosperm texture is one of the primary determinants of milling performance and end-product quality in wheat. A single major gene (*Ha*) controls this trait (Symes 1965) and promotes softness or inhibits hardness through some unknown biochemical means (Law and Krattiger 1987). Greenwell and Schofield (1986) reported the existence of a starch granule protein with a molecular weight of 15 kDa (friabilin) (Greenwell and Schofield 1989) that is either a gene product under the control of this hardness locus (and therefore a pleiotropic marker for or an effector of endosperm softness) or a separate but tightly linked gene. This distinction, a single gene or two separate but tightly linked genes, is central to advancing our understanding of the control of endosperm texture in wheat.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate starch granule proteins and thereby correlate the occurrence of friabilin with endosperm texture. SDS-PAGE also permitted the quantitation of friabilin through the use of densitometric analysis of stain intensity (Schofield and Greenwell 1987, Greenwell and Schofield 1989, Sulaiman and Morrison 1990).

During our research on friabilin isolated after the method described by Greenwell and Schofield (1986), we experienced gel-to-gel inconsistencies in friabilin staining intensity and its resolution in SDS-PAGE. We discovered that these problems resulted from the solubility of friabilin in the methanol-water-acetic acid mixture, which commonly is used for protein fixation (in situ precipitation) in polyacrylamide gels. Figure 1 shows that a dramatic reduction in friabilin staining intensity occurred after an overnight incubation in a mixture of methanol, water, and acetic acid (lane D) compared with trichloroacetic acid (lane B). Restaining of gels indicated that the protein had been lost. With extended incubation (e.g., 3 days), however, stain intensity of the band at the same mobility as friabilin in the soft wheat lane did not change, such that staining intensity was essentially the

same as that shown in Figure 1, lane D. In the hard wheat samples, a faint band of mobility similar to friabilin was present (lane C, barely visible in lane A). SDS-PAGE gels other than those presented here suggested that this band was apparently not soluble in a methanol-water-acetic acid fixative (no change in stain intensity).

By changing the acid component of the fixative solution to trichloroacetic acid, the loss of friabilin was eliminated (lane B), as were the previously described problems with inconsistent staining, including band diffusion. With this fixative, friabilin stain intensity in gels fixed and stained immediately after electrophoresis was comparable to the intensity of gels fixed for 2 days and then stained (data not shown). This result was independent of the type of stain used, and both silver and Coomassie Blue staining produced similar results.

A question remained regarding the identity of the 15-kDa band, which apparently was not soluble in the methanol-water-acetic acid fixative and was present in the soft and hard wheat lanes (Fig. 1, lanes C and D). To address this question, we used the SDS-PAGE buffer system of Schagger and von Jagow (1987). In contrast to the Laemmli system (Laemmli 1970), which uses glycine as the trailing ion, this system uses tricine and is reported to provide improved separation in the range of 5–20 kDa. Our results show that individual band resolution was actually poorer in this system (Fig. 1, lanes E–H), although friabilin was similarly well separated from other major bands (lane F). With this system, however, it was clear that friabilin was completely lost from the gel with incubation in the methanol-water-acetic acid mixture (lane H), and no band remained as in the Laemmli system (compare lane D). The Schagger and von Jagow tricine gel system, however, provided no additional insight as to the existence of friabilin in hard wheat.

The separation capability of the SDS-PAGE/tricine system, coupled with the selective solubilization capability of the methanol-water-acetic acid fixative, has been used to electrophoretically isolate pure friabilin from soft wheat starch granules. In this procedure, the portion of the gel containing friabilin is excised, and friabilin is eluted with the methanol-water-acetic acid fixative and recovered via acetone precipitation and subjected to electrophoresis to produce a single band (data not shown). Using hard wheat starch, this isolation procedure produces a very faint 15-kDa band and, therefore, provides support (Greenwell and Schofield 1986, Bakhella et al 1990) that friabilin occurs in hard wheat as well.

In summary, these results suggest that compared to most wheat

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proteins and, in particular, other starch granule proteins, friabilin has the unique property of being soluble in the common PAGE fixative of methanol, acetic acid, and water. Second, results also

suggest that previous results that used SDS-PAGE and methanol-water-acetic acid fixation to examine the occurrence of friabilin must be carefully reexamined, particularly those that employed densitometric analysis. And third, the separation of starch proteins by SDS-PAGE and the elution of friabilin from the gel with a methanol-acetic acid-water fixative provide a novel and efficient method of isolating relatively pure friabilin.

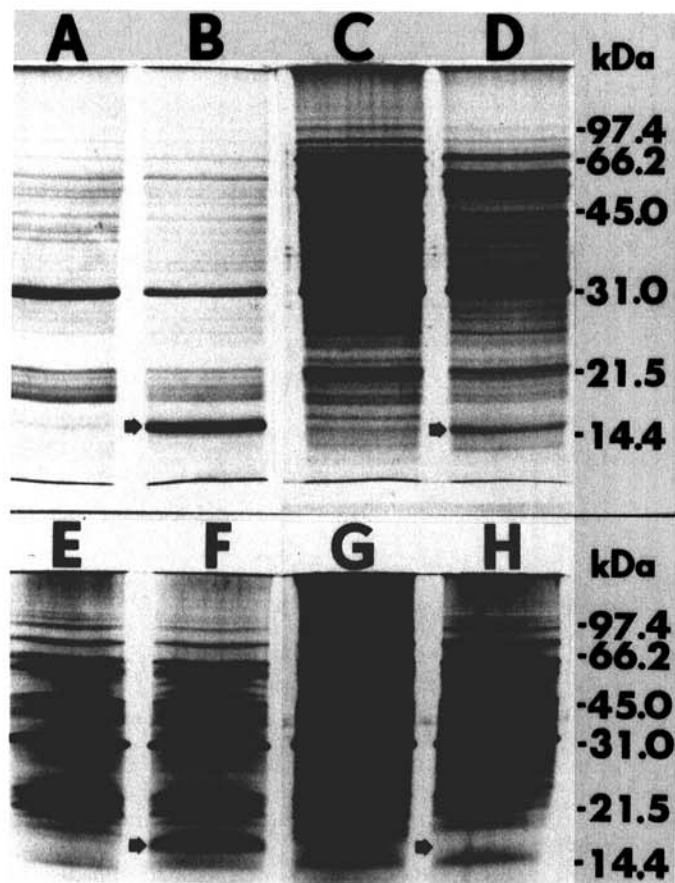


Fig. 1. SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of starch granule proteins. Starch granule proteins from hard wheat cv. Butte 86 and soft wheat cv. Lewjain were electrophoresed in the discontinuous SDS-PAGE buffer system of Laemmli (1970) (lanes A–D) or Schagger and von Jagow (1987) (lanes E–H). Gels were subdivided and the appropriate portion was incubated either in a methanol-water-acetic acid fixative (50:40:10) (lanes C, D, G, and H) or a methanol-water-trichloroacetic acid fixative (45:43:12) (lanes A, B, E, and F) and silver stained after the method of Morrissey (1981). The right portion of each gel (incubated in methanol, water, and acetic acid) is overstained relative to the left portion to demonstrate the presence or absence of friabilin. Lanes A, C, E, and G are hard wheat starch granule proteins, and lanes B, D, F, and H are soft wheat starch granule proteins. Arrows indicate the presence or absence of friabilin (15 kDa molecular weight) in the soft wheat extracts.

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