PROTEINS

Two-Dimensional Electrophoretic Analysis of Friabilin

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Friabilin, a 15-kDa protein, was found to be associated with a soft endosperm texture (Greenwell and Schofield 1986). In Japanese wheat cultivars, a highly significant correlation was also demonstrated between the concentration of friabilin and the flour particle size (Oda et al 1992). Using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), friabilin was detected as one band at 15 kDa. Preliminary isoelectric focusing (IEF) results indicated that friabilin comprised a single protein with a pI of ~7.5–8.0 (Greenblatt et al 1992). This study used two-dimensional electrophoresis to analyze friabilin.

MATERIALS AND METHODS

Purified starch of wheat (cv. Fukuho Komugi) was used. Starch granule proteins were extracted by the method of Greenwell and Schofield (1986). Prime starch was suspended in a 1% (w/v) SDS solution and shaken at 50°C for 1 hr. The slurry was centrifuged at 15,000 × *g* for 5 min. The resulting supernatant was concentrated by precipitation with cold acetone. The precipitation was performed in a freezer at −20°C overnight. Precipitates were collected by centrifugation at 15,000 × *g* for 10 min and dissolved directly in lysis buffer (O'Farrell 1975) without 2-mercaptoethanol for one-dimensional electrophoresis.

Nonequilibrium pH gradient electrophoresis (NEPHGE) was used in the first dimensional electrophoresis. Pharmalyte (Pharmacia) with a range of pH 3–10 was used for NEPHGE. The NEPHGE rod gels were 6 cm long and 1 mm in diameter. The gel mixture was 3.5% acrylamide, 8*M* urea, 2% nonidet P-40 (NP-40), and 5% pharmalyte. NEPHGE was performed for 600 V·hr with 0.02*M* NaOH (cathodic solution) and 0.01*M* H_3PO_4 (anodic solution). The gels were equilibrated and fixed for 15 min in 45% methanol and 12% trichloroacetic acid. The gels were then rinsed with water.

Tricine-SDS-PAGE was used in the second dimensional electrophoresis (Schägger and von Jagow 1987). The gel was composed of separation gel (16.5%T, 3%C), spacer gel (10%T, 3%C), and stacking gel (4%T, 3%C).

The protein bands were stained with 0.1% (w/v) coomassie brilliant blue R-250 in 50% (v/v) methanol and 10% (v/v) acetic acid. A background destaining was achieved by shaking the gel in 25% (v/v) methanol and 7.5% (v/v) acetic acid.

RESULTS AND DISCUSSION

Figure 1 shows two-dimensional electrophoresis gel of starch granule proteins. Arrows indicate friabilin spots. Friabilin was divided into two major spots and two minor ones in this two-dimensional electrophoresis. The most basic spot of friabilin was the most basic protein in starch granule proteins.

The protein with a pI of ~7.5–8.0 (Greenblatt et al 1992) may be the most acidic spot of friabilin in Figure 1. The other spots of friabilin may be moved out of a gel under the IEF conditions of Greenblatt et al (1992).

Recently, some reports (Blochet et al 1993, Greenwell and Brock 1993, Jolly et al 1993) showed that friabilin was not one polypeptide, but a mixture of polypeptides. This two-dimensional electrophoresis result is consistent with those reports.

I also analyzed endosperm proteins of hard wheat (Avalon) and soft wheat (Galahard) by this two-dimensional electrophoresis. However, friabilin was detected in both hard and soft wheats, and there was no difference in friabilin between hard and soft wheat (data not shown). Jolly et al (1993), using an antiserum specific for friabilin, showed that friabilin accumulated in both hard and soft wheat grains. This two-dimensional electrophoresis result is consistent with results of Jolly et al (1993). These results indicate that the structure gene for friabilin and the softness-hardness locus (*Ha*) may be located very close to each other. The *Ha* gene may affect the friabilin accumulation on starch surface or interaction between starch surface and friabilin. However, to elucidate genetically whether the structure gene for friabilin and *Ha* are a single gene or whether they are located very close to each other, varietal variation for friabilin in endosperm proteins is necessary. If there is varietal variation for friabilin in endosperm proteins, we can perform genetic analysis of the structure gene for friabilin and *Ha*.

Friabilin-like proteins were found in rye and triticale (Schofield et al 1991) and in *Aegilops* and *Triticum* diploid species (Morrison et al 1992). These results showed that there must be a structure gene for friabilin in the A, B, D, and R genomes. Morrison et al

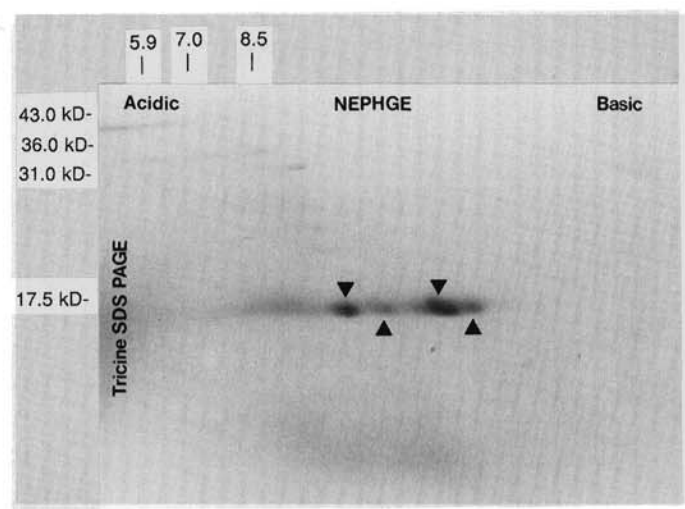

Fig. 1. Two-dimensional electrophoresis of friabilin. Starch granule proteins from the soft wheat cv. Fukuho Komugi were electrophoresed in two-dimensional electrophoresis; the first dimensional electrophoresis was nonequilibrium pH gradient electrophoresis (NEPHGE); the second dimensional electrophoresis was tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (Tricine-SDS-PAGE). Arrows indicate friabilin spots. Two-dimensional SDS-PAGE standards were used for pI markers and molecular mass markers.

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(1992) suggested that these friabilins may not be identical and should be designated friabilin-A, friabilin-B, friabilin-D, and friabilin-R to identify the genome carrying the structure gene. The two-dimensional electrophoresis will be useful in revealing whether or not friabilin-A, B, D, and R are different.

Using one-dimensional electrophoresis, friabilin was detected as one protein band. However, using two-dimensional electrophoresis, friabilin was divided into at least four spots. Therefore, to elucidate the function of friabilin, the spots were purified and analyzed separately. The two-dimensional electrophoresis method will be useful in elucidating the function of friabilin and the mechanism of endosperm texture.

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