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## Immunological characterization of $\alpha$ -amylase isozymes in developing and germinating wheat seeds

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$\alpha$ -Amylase isozymes from embryos and endosperms of germinating wheat seeds were analyzed by isoelectric focusing. The isozymes were divided into two groups, group I with isoelectric points ranging from pH 6.0 to 6.5 and group II from pH 4.5 to 5.0. In developing seeds, only the group II isozymes were found, while they disappeared in the embryos at the first and second days of germination. Two isozyme groups were separated by preparative isoelectric focusing and examined by the double immunodiffusion method using anti- $\alpha$ -amylase antiserum. It was found that the isozyme groups were different each other immunologically.

$\alpha$ -Amylase in cereals has been shown to exist in isozymic forms (SCANDALIOS, 1974). The compositions of  $\alpha$ -amylase isozymes vary in the species of cereals and the stages of development or germination of seeds (FRYDENBERG and NIELSEN, 1965; TANAKA *et al.*, 1970; DAUSSANT and RENARD, 1972). In wheat seeds, fifteen isozymes were detected by isoelectric focusing and divided into two groups. The genetic analysis demonstrated that the structural genes of two groups of the isozymes located on different chromosomes (NISHIKAWA and NOBUHARA, 1971).

On the other hand, immunochemical studies with anti- $\alpha$ -amylase immune serum showed the antigenic differences in maturing and germinating wheat seeds (DAUSSANT and RENARD, 1972). In a previous report, we showed that the  $\alpha$ -amylase of germinating wheat seeds was separated into two components by immunoelectrophoresis (FUJIOKA *et al.*, 1984). In this study,  $\alpha$ -amylase isozymes of both embryos and endosperms from germinating wheat seeds were analyzed by isoelectric focusing. The immunological differences were found in two groups of the isozymes.

### Materials and Methods

Winter wheat seeds (*Triticum aestivum* L. cv. Mukakomugi) were ster-

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ilized in 0.05%  $\text{HgCl}_2$  solution for 15 min, rinsed with running water and germinated in the dark at 24°C. Seeds and seedlings in each stage of germination were separated into embryos and endosperms. Developing seeds were isolated from ears collected at the second week after flowering from experimental plots.

The samples frozen at -70°C were homogenized in a chilled mortar and pestle with two volumes of the extraction medium containing 0.1 M sodium acetate buffer (pH 4.8), 20 mM  $\text{CaCl}_2$  and 5 mM 2-mercaptoethanol. The homogenate was centrifuged at  $3,000 \times g$  for 15 min. The supernatant solution was heated at 70°C for 15 min and centrifuged at  $10,000 \times g$  for 10 min. The resultant supernatant was concentrated by the ultrafiltration.

Isoelectric focusing was performed in a slab gel contained 5% acrylamide and 2.4% Ampholine (LKB) in the pH range 4.5-7.0. Enzyme solutions were loaded on the surface of the gel, the gel was electrophoresed for 3 h at 10°C and 15 W constant power. After electrophoresis, the gel was placed on the glass coated with a thin starch-polyacrylamide film (7.5% acrylamide, 1% starch). After incubation at 37°C for 15 min, the gel was removed and the starch-film-glass was immersed in an acidic  $\text{I}_2$ -KI solution.

Preparative electrophoresis of a granulated gel in flat-bed was used to prepare the isozymes of group I and II apart. The gel was contained 6.6% Sephadex G-75 (Superfine) and 2% Ampholine in a pH range 5.0-7.5. Enzyme solution was mixed with small amount of the gel and the mixture was applied to the gel bed. After electrophoresis at 10°C and 8 W constant power for 15 h, the gel bed was separated with the fractionating grid and each gel section was eluted with the extraction medium.

Antibody against  $\alpha$ -amylase was obtained from rabbits immunized with the wheat  $\alpha$ -amylase purified from 3-day germinated seedlings as described in a previous report (FUJIOKA *et al.*, 1984).

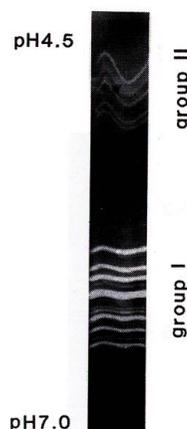
Amylase activity was measured by the modified blue value method of FUWA (1954).

## Results and Discussion

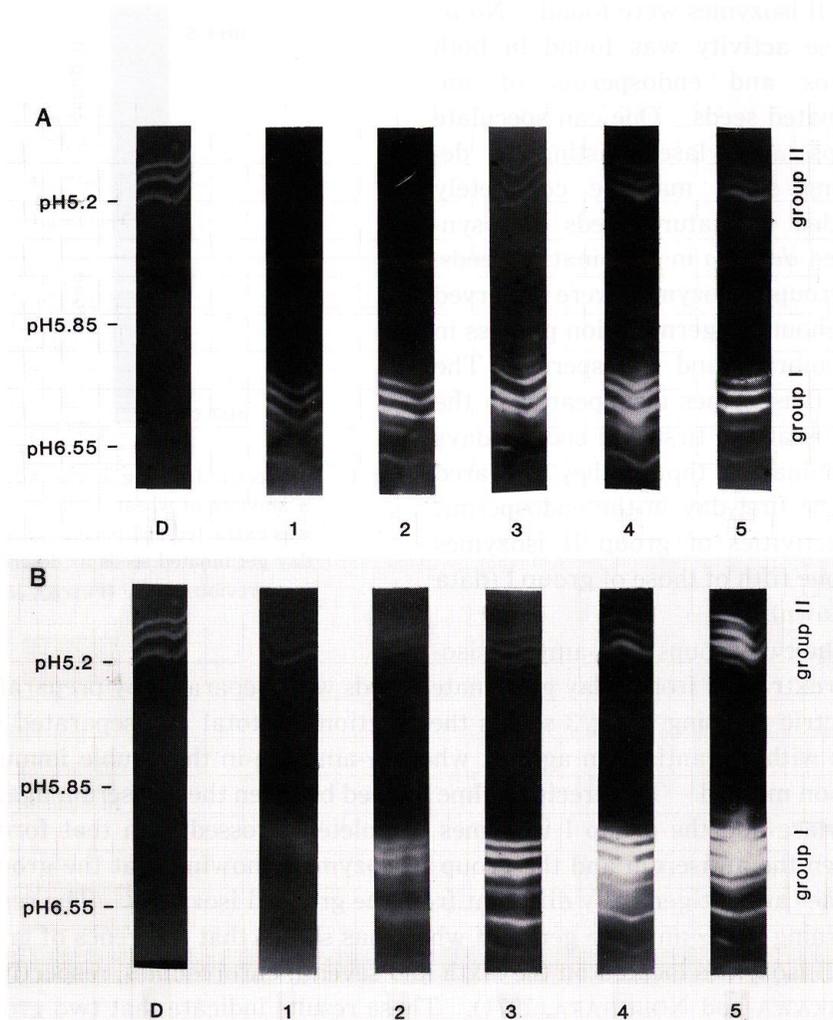
As shown in Fig. 1, the purified  $\alpha$ -amylase of wheat was separated into two groups by isoelectric focusing. The enzyme group with the isoelectric points ranging from pH 4.5 to 5.0 containing five isozymes was designated group II and that ranging from pH 6.0 to 6.5 containing twelve isozymes was designated group I. Changes in  $\alpha$ -amylase isozymes of developing and germinating wheat seeds are shown in Fig. 2. In developing seeds, only

group II isozymes were found. No  $\alpha$ -amylase activity was found in both embryos and endosperms of ungerminated seeds. One can speculate perhaps  $\alpha$ -amylase existing in developing seeds may be completely degraded in mature seeds and synthesized *de novo* in germinating seeds. The group I isozymes were observed throughout the germination process in both embryos and endosperms. The group II isozymes disappeared in the embryos at the first and second days of germination, though they appeared from the first day in the endosperms. The activities of group II isozymes were one fifth of those of group I (data not shown).

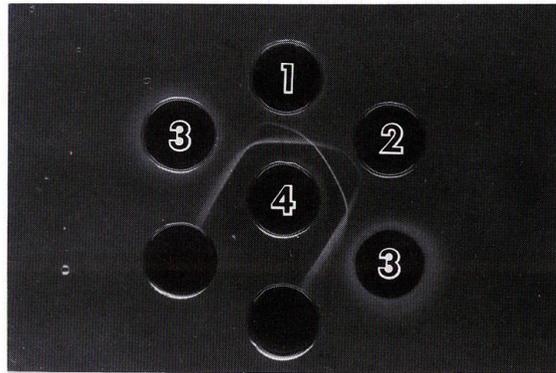
The two groups of  $\alpha$ -amylase isozymes extracted from 3-day germinated seeds were separated by preparative isoelectric focusing. Fig. 3 shows the reactions of total and separated isozymes with the antiserum against wheat  $\alpha$ -amylase in the double immunodiffusion method. The precipitin line formed between the antiserum against  $\alpha$ -amylase and the group I isozymes completely crossed with that formed between the antiserum and the group II isozymes, showing that the group I isozymes are antigenically different from the group II isozymes. The report concerning the  $\alpha$ -amylase genes of wheat has shown that the genes of group I and II isozymes located on the sixth and seventh chromosomes, respectively (NISHIKAWA and NOBUHARA, 1971). These results indicate that two groups of the isozymes are synthesized from the mRNAs transcribed from different genes, though it has been shown that  $\alpha$ -amylase mRNAs in mouse salivary gland and liver were coded by the same gene but contained different sequences (YOUNG *et al.*, 1981). The existence of two groups in  $\alpha$ -amylase isozymes was shown in barley, and the serological difference was also shown between the two groups (JACOBSEN and HIGGINS, 1982). The control mechanisms in the synthesis of  $\alpha$ -amylase isozymes including the effects of a gibberellin have to be elucidated in the embryos and the endosperms of wheat seeds.



**Fig. 1** Isoelectric focusing of the purified  $\alpha$ -amylase of wheat. The enzyme was extracted and purified from 3-day germinated seeds as described in a previous report (FUJIOKA *et al.*, 1984).



**Fig. 2** Isoelectric focusing of  $\alpha$ -amylase from developing and germinating wheat seeds. **A.**  $\alpha$ -Amylase isozymes of developing seeds and of embryos from germinating seeds. Lane D: developing seeds, lanes 1 to 5: embryos from 1-, 2-, 3-, 5- and 7-day germinated seeds, respectively. **B.**  $\alpha$ -Amylase isozymes of developing seeds and of endosperms from germinating seeds. Lane D: developing seeds, lanes 1 to 5: endosperms from 1-, 2-, 3-, 5- and 7-day germinated seeds, respectively. The experimental conditions are described in text.



**Fig. 3** Double immunodiffusion analysis of two groups of  $\alpha$ -amylase isozymes. 1: group I isozymes of  $\alpha$ -amylase, 2: group II isozymes of  $\alpha$ -amylase, 3: total  $\alpha$ -amylase, 4: antiserum against  $\alpha$ -amylase of wheat.  $\alpha$ -Amylase was extracted from 3-day germinated seeds of wheat and separated into two groups by isoelectric focusing as described in text.

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