Short Communication

Isozymes of α-Amylase Induced by Gibberellic Acid in Embryo-less Grains of Barley

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Yomo (8) and Paleg (5) independently found that the induction of α-amylase in the embryo-less grain was stimulated by gibberellin. This fact was confirmed by several investigators (1,4,7). Recently one of the authors and his co-workers found that helminthosporal and its air-oxidation derivative, helminthosporic acid, had the same effect as gibberellin. We have investigated isozyme(s) of α-amylase induced by gibberellic acid and helminthosporal to see whether or not the gibberellin- and helminthosporol-induced α-amylase are the same. This paper reports on isozymes of α-amylase induced by GA₃.

Seeds of Hordeum distichon L., Kirin-chokuichi, of the 1965 harvest were used in this experiment. Seeds were dehulled with 50% (v/v) sulfuric acid and washed with running water. Embryos were removed from the seeds with a razor blade. The remaining endosperms were soaked in 75% alcohol (v/v) for 30 seconds and sterilized by dipping into a supernatant solution of 10% concentration of bleaching powder solution (g/v) for 10 minutes. After rinsing with sterile distilled water, 40 endosperms were placed in a 9 cm petri dish which contained either 10 ml of sterile distilled water or the same volume of 2 μM GA₃ solution. The petri dishes were incubated at 25° for 4 days.

One hundred endosperms were extracted with calcium acetate-sodium chloride solution [0.01 m Ca(O-CO-CH₃)₂ - 0.02 m NaCl]. The extract was precipitated by adding solid ammonium sulfate up to 0.75 saturation. The precipitate was centrifuged at 0°, the supernatant fraction being discarded. The precipitate was dissolved in a small volume of 0.01 m Tris-HCl buffer containing 0.01 m NaCl, pH 8.5, and filtered through Sephadex G 50 column, 2 × 20 cm, to eliminate low molecular compounds. The filtrate was adsorbed onto a 2 × 20 cm DEAE-cellulose column. The active material was eluted with 200 ml of solution, employing a linear gradient of 0.01 to 1.00 m NaCl. The NaCl solution was adjusted to pH 8.5 with 0.01 m Tris-HCl buffer. Eluate was collected for each 4 ml and the amylase activity was determined as follows. But the culture medium was not used to extract, because the medium contained only a small amount of the α-amylase which was similar chromatographic pattern of the endosperm.

The activity of α-amylase was measured by the Blue value method modified by Fuwa (3). Two ml of 0.1 m acetate buffer, pH 5.7, which contained 0.5% starch, was added to 0.5 ml of enzyme solution. After 15 or 30 minutes of incubation of the combined solution at 40°, 5 ml of 0.5 N acetic acid was added. One ml of this solution was added to 10 ml of iodine solution which consisted of 0.0003 M iodine, a small amount of potassium iodide and 0.03 N HCl. The optical density of this solution at 700 μm was measured at room temperature with a Hitachi-spectrophotometer. Activity unit of the enzyme was expressed as μg of hydrolyzed starch under the condition in which the optical density of starch-iodine complex at 700 μm was decreased 10% with 1 ml of enzyme solution for 30 minutes at 40°. The activity of β-amylase was measured by the method of Schwimmer (6).

Three different fractions with α-amylase activity were obtained. We tentatively named the fractions α-amylase-I (α₁), -II (α₂) and -III (α₃) according to the order of elution (fig 1). The α₃ fraction induced by 2 μM GA₃ showed a high activity of α-amylase. The amount of α₃ was greater than that of both α₁ and α₂. But in the case of treatment with higher concentration of GA₃, such as 0.1 mm helminthosporal, the amount of α₃ was too small to be isolated. According to our data for 0.2 mm helminthosporal, the amount of α₃ was about twice that of α₂. Each amount of α₁ and α₂ induced by 2 μM GA₃ was more than 5 times that of the control. Although the α₃ fraction was not obtained in the control. Varner showed only 1 fraction with α-amylase activity (7). On the other hand, we obtained 3 active fractions at least. The difference between Varner’s result and ours may be due to the difference of incubation period and pH value of eluant. GA₃ had no effect on the level of β-amylase.

Each of the 3 fractions induced by GA₃ was concentrated to a small volume in an ice box and dialysed for 2 days against 0.01 m Tris-HCl buffer, pH 8.5, which contained 0.01 m NaCl. After the dialysis, each of the fractions was rechromato-
Fig. 1. Chromatographic patterns of amylases in the embryo-less grain of barley treated with 2 μM GA₃ and a rechromatographic pattern of the α₂ fraction (---). Ordinate (left): Activity of α-amylase (——). But the scale for the α₂ signify one-fifth of the ordinate scale. Ordinate (right): Amount of reducing sugar released by β-amylase as expressed in mg of K₃Fe(CN)₆ (——). obtained a considerable amount of the α₁ in the half endosperm with embryo 1 or 2 days after the treatment with sterile water. The α₂ was also obtained after 3 days and the α₃ did after 4 days. Such a process was observed in the case of embryo-less grains treated with 2 μM GA₃. A similarity in the processes between the GA₃ treatment and the half endosperm with embryo may be explained from the result obtained by Yomo (9).

Behaviors on electrophoresis, thermal stabilities and kinetic properties of the α₁, α₂, and α₃ induced by GA₃ will be discussed later.

Literature Cited