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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 helminthosporol 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 helminthosporol 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 GA_3.

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 in a supernatant solution of 10 % concentration of bleaching powder solution (g/v) for 10 minutes. After rinsing with sterile redistilled water, 40 endosperms were placed in a 9 cm petri dish which contained either 10 ml of sterile redistilled water or the same volume of 2 μM Ga_3 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 $Ca(O\text{-}CO\text{-}CH_3)_2$ − 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 mμ was measured at room temperature with a Hitachi-spectrophotometer. Activity unit of the enzyme was expressed as mg of hydrolyzed starch under the condition in which the optical density of starch-iodine complex at 700 mμ 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 (α_1), -II (α_2) and -III (α_3) according to the order of elution (fig 1). The α_1 fraction induced by 2 μM GA_3 showed a high activity of α-amylase. The amount of α_2 was greater than that of both α_1 and α_3. But in the case of treatment with higher concentration of GA_3, such as 0.1 mM helminthosporol, the amount of α_3 was too small to be isolated. According to our data for 0.2 mM helminthosporol, the amount of α_3 was about twice that of α_2. Each amount of α_1 and α_2 induced by 2 μM GA_3 was more than 5 times that of the control. Although the α_3 fraction was not obtained in the control. Varner showed only 1 fraction with α-amaylase 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 elutant. GA_3 had no effect on the level of β-amaylase.

Each of the 3 fractions induced by GA_3 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-

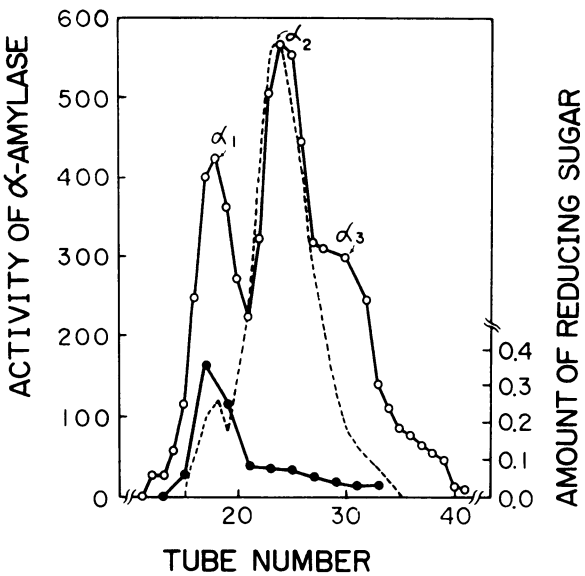

FIG. 1. Chromatographic patterns of amylases in the embryo-less grain of barley treated with 2 μM GA_3 and a rechromatographic pattern of the α_2 fraction (- - - -). Ordinate (left): Activity of α-amylase (——○——). But the scale for the α_2 signify one-fifth of the ordinate scale. Ordinate (right): Amount of reducing sugar released by β-amylase as expressed in mg of $K_3Fe(CN)_6$ (——●——).

graphed on a DEAE-cellulose chromatogram. Each fraction was found to be eluted at positions corresponding to those mentioned above. The rechromatographic pattern of the α_2 fraction is shown as a broken line in figure 1.

Frydenberg and Nielsen separated 5 isozymes of α-amylase in the germinating barley (2). In their experiment, they obtained 1 band with a high enzyme activity on the third day after germination and 5 bands, of which 2 had higher activity than the others, on the sixth day. In our case, we obtained a considerable amount of the α_1 in the half endosperm with embryo 1 or 2 days after the treatment with sterile water. The α_2 was also obtained after 3 days and the α_3 did after 4 days. Such a process was observed in the case of embryo-less grains treated with 2 μM GA_3. A similarity in the processes between the GA_3 treatment and the half endosperm with embryo may be explained from the result obtained by Yomo (9).

Behaviors on electropholesis, thermal stabilities and kinetic properties of the α_1, α_2, and α_3 induced by GA_3 will be discussed later.

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