Short Communication

Purification and Action Pattern on Soluble Starch of α -Amylase from Sugar Cane Leaves

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α -Amylase [EC 3.2.1.1] is ubiquitous in living cells, and is important for the use of polysaccharides *in vivo*. In higher plants, α -amylases have been isolated from barley,^{1,2)} oats,^{1,2)} rye,^{1,2)} wheat,^{1,2)} soybeans,^{1,2)} broad beans,^{1,2)} rice,³⁾ and sorghum⁴⁾ and extensive studies have been done on their properties and structures. We have been studying the amylases of sugar cane leaves, and found a novel type of α -amylase producing maltoheptaose as the main product from soluble starch. In this communication, we describe the isolation and the partial properties of this α -amylase from sugar cane leaves.

The amylase activity was measured as follows. The reaction mixture, consisting of 0.5 ml of enzyme solution and 0.5 ml of 2% soluble starch in a 50 mM acetate buffer (pH 5.0) containing 1 mM CaCl_2 , was incubated at 35°C for an appropriate time and the reducing sugar produced was measured by the Somogyi⁵⁾–Nelson⁶⁾ method with glucose as the standard. One unit of the enzyme activity was defined as the amount of enzyme that liberated reducing sugar equivalent to 1 μmol of glucose per minute under the above conditions. Protein was measured by the method of Bradford,⁷⁾ using bovine serum albumin as a standard protein. Sugar cane leaves were homogenized in 500 ml of 50 mM acetate buffer (pH 5.0) containing 1 mM CaCl_2 per 50 g of leaves' weight with a blender (Matsushita Electric Co., MX-150). The crude homogenate

was then filtered through bleached cotton to remove gross particulate matter and centrifuged at 8,000 rpm for 30 min. The supernatant (500 ml) was put on an α -cyclodextrin Sepharose 6B column (3.0 $\times$ 10 cm), which was prepared by the method of Uy and Wold,⁸⁾ equilibrated with 50 mM acetate buffer (pH 5.0) containing 1 mM CaCl_2 . After washing with 300 ml of the same buffer, the adsorbed proteins were eluted with 200 ml of the same buffer containing α -cyclodextrin (2 mg/ml). Two distinct active protein peaks, an unadsorbed peak (peak I) and an adsorbed peak (peak II) on the affinity column, were obtained. Peak II fractions were then pooled and lyophilized. The lyophilized materials were dissolved in 10 ml of cold water and gel-filtered on a Sephadex G-100 column (4.0 $\times$ 120 cm) equilibrated with the same buffer. The amylase activity fractions showed a symmetrical peak were obtained. This preparation showed a single band of protein on a disk gel electrophoresis (pH 9.4) by the method of Davis,⁹⁾ and used in the following experiments as purified enzyme. In these purification steps, about 7.8% of the amylase was recovered with over a 80-fold increase in specific activity.

The enzyme solution of 0.5 ml (0.04 U) was incubated with 0.5 ml of 4% soluble starch in 50 mM acetate buffer (pH 5.0) containing 1 mM CaCl_2 at 50°C for 0–14 hr and then boiled for 10 min to stop the reaction, and an appropriate amount of the solution was used for HPLC on a Shimadzu-Pack CLC-NH₂ column (0.6 $\times$ 15 cm). Products were identified by comparison with the retention times of authentic samples (G_1 – G_8). Products were measured by the peak area. Figure 1 shows the course of hydrolysis of soluble starch by this amylase. This clearly showed that G_7 was produced preferentially during all stages of hydrolysis at the maximum yield of 25% although G_1 – G_6 were also observed in the reaction mixture, and that G_8 gradually decreased after reaching a plateau. This result suggests that this enzyme is an endo-type amylase which can not readily hydrolyze maltooligosaccharides smaller than G_7 .

Fig. 1. Course of Hydrolysis of Soluble Starch by the Amylase.

Oligosaccharides produced in the course of the enzymatic reaction were examined by HPLC with a differential refractometer (RID-6A, Shimadzu Seisakusho Co.) as a detector, on a Shimadzu-Pack CLC-NH₂ column (0.6 × 15 cm) under the following conditions: elution with acetonitrile-water (6:4, v/v); flow rate, 1.0 ml/min; and column temperature, 40°C.

Symbols: (—○—), glucose (G₁); (—●—), maltose (G₂); (—□—), maltotriose (G₃); (—■—), maltotetraose (G₄); (—△—), maltopentaose (G₅); (—▽—), maltohexaose (G₆); (—▲—), maltoheptaose (G₇); (—▼—), maltooctaose (G₈).

The mutarotation of the products from soluble starch with the enzyme was examined. The reaction was started by mixing 2.5 ml of the enzyme solution (0.2 U) with 2.5 ml of 2% soluble starch in 50 mM acetate buffer (pH 5.0) containing 1 mM CaCl₂ at room temperature in a cuvette (light path, 5 cm) placed in an automatic digital polarimeter (Nihon Bunkou DIP-180), and the change in optical rotation at 589 nm was recorded. After 15 min, one drop of 25% ammonia water was added to stop the reaction. The optical rotation was observed to decrease from 0.757 to 0.740 degrees after the addition of ammonia water. This indicates that the enzyme produced α -anomer products from soluble starch. Consequently, we concluded that this amylase was a novel type of α -

amylase producing maltoheptaose as the main product from soluble starch.

More detailed investigations on this work will be reported later.

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