Note

Changes in Activities of Starch-hydrolyzing Enzymes in Roots of Sugar Beets

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In our previous paper,¹⁾ we reported that mature roots of sugar beets contained starch-hydrolyzing enzymes such as glucoamylase, α -amylase, and debranching enzyme, though starch was not detected. We are interested in whether or not these enzymes are involved in the metabolism of sucrose or other sugars. In this paper, we describe the changes in activities of starch-hydrolyzing enzymes during root growth of sugar beets.

The sugar beet roots used were the same as those described in the previous paper.²⁾ In experiments of the changes in the activities of starch-hydrolyzing enzymes in roots during plant growth, the enzymes were prepared by the procedures described in a previous paper²⁾: Roots (50 g fresh weight), harvested at 15-day intervals, were homogenized with three volumes of 10 mM phosphate buffer (pH 7.4) containing 2% sodium isoascorbate in a Waring blender. The filtrate was treated with solid ammonium sulfate between 20 and 80% saturation and centrifuged at $12,000 \times g$ for 20 min. The fraction that precipitated was dissolved in a small amount of 5 mM phosphate buffer (pH 7.4) containing 5 mM β -mercaptoethanol, and dialyzed overnight against the same buffer. After centrifugation of the dialyzed solution to remove the insoluble matter, the supernatant was put on a DEAE-cellulose column (1.9 $\times$ 40 cm), pre-equilibrated with 5 mM phosphate buffer (pH 7.4) containing 5 mM β -mercaptoethanol. The column was first washed with the same buffer to remove the unadsorbed proteins, and then eluted with a linear gradient from 0 to 0.8 M NaCl in 500 ml of the buffer. Three types of starch-hydrolyzing enzyme were well separated by DEAE-cellulose chromatography, as described in our previous paper.¹⁾

The activities of α -amylase, glucoamylase, and debranching enzyme were assayed as follows: The reaction mixture consisted of 0.25% soluble starch or pullulan, 20 mM citrate buffer (pH 4.4 for glucoamylase, pH 6.8 for α -amylase, and pH 5.6 for debranching enzyme) and 300 μ l of enzyme solution in a final volume of 1 ml.

The mixture was incubated at 37°C for 30 min. The amount of reducing sugar liberated was measured by the method of Somogyi and Nelson.³⁾ One unit of amylase or debranching enzyme was defined as the amount of enzyme which catalyzed the production of 1 μ mol of reducing sugar as glucose for glucoamylase, maltose for α -amylase, and maltotriose for debranching enzyme, for 30 min at 37°C.

Figure 1 shows the changes in the activities of three starch-hydrolyzing enzymes during development and after harvesting of the root. α -Amylase, glucoamylase, and debranching enzyme all appeared at 52 days when roots began to enlarge and store sucrose, and then increased in their activities till 112 days when sucrose reached a

FIG. 1. Changes in the Activities of Glucoamylase, α -Amylase, and Debranching Enzyme of Sugar Beet Roots during Growth and Storage.

(●), glucoamylase; (○), α -amylase; (■), debranching enzyme.

FIG. 2. pH-Activity Profiles of α -Amylase in Roots and Leaves of Mature Plants.

α -Amylase in roots and leaves were prepared by procedures including ammonium sulfate fractionation and DEAE-cellulose chromatography.

(○), root α -amylase; (●), leaf α -amylase.

constant level, followed by a decline. Their activities remained constant during root storage after harvest. The pattern of the changes in activities of three enzymes during root development was nearly the same as that of alkaline invertase. Alkaline invertase is suggested to be an important regulatory enzyme in sucrose accumulation, because the enzyme activity was not detected in immature tissues and appeared when the roots began to accumulate sucrose, and thereafter increased with the progress of sucrose accumulation.²⁾ This suggested that root enlargement was accompanied by sucrose accumulation and simultaneous induction of starch-hydrolyzing enzymes and alkaline invertase.

On the other hand, in leaves of sugar beet, glucoamylase was hardly detectable in both 4-week-old young and mature plants, and debranching enzyme was present in

young but not in mature plants. α -Amylase activity was high in young plants but very low in mature ones. Thus leaves of mature plants contained only α -amylase of the three amylolytic enzymes which were detected in roots. The pH-activity profile of α -amylase in mature roots differed from that in leaves: the enzyme in roots had a narrow range in optimum pH while the leaf one had a broader range, as shown in Fig. 2.

REFERENCES

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