Note

Starch-hydrolyzing Enzymes in Germinating Kidney Bean

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Kidney bean (Phaseolus vulgaris L. cv. Toramame) seeds have α-glucosidase and α-amylase activities during the germination stage. No detectable α-glucosidase activity was found in dry seeds or during the short period of imbibition, unlike other plant seeds.1-3 Both activities of α-glucosidase and α-amylase reached maxima at about 8-9 d of germination. To test whether or not α-glucosidase and α-amylase are synthesized de novo, a density labeling method was used on the seeds grown with either H2O or D2O. No density shift was observed in α-glucosidase activity. By contrast, α-amylase showed different densities between H2O- and D2O-germinated seed preparations. These results imply that α-glucosidase activity of kidney bean might be due to activation of a pre-existing form, while the α-amylase is formed using not free amino acid pools but the amino acids synthesized or derived by hydrolysis of proteins.

Kidney bean seeds, kindly supplied from Hokuren (Sapporo), were soaked overnight in filtered tap water with a 0.45-μm membrane filter, and were put on a small drainboard in a plastic tray. During germination at 27°C in a dark chamber, water was supplied to be always kept under the bottom of the drainboard. Three seedlings were harvested at regular intervals, and their cotyledons were homogenized in 0.1 m sodium acetate buffer, pH 6.0, containing 5 mm CaCl2 (1.5 ml/cotyledon) with a Polytron (Kinematica GmbH), and stirred for 3 h. The supernatant was obtained by centrifugation at 16,000 × g for 15 min, and then the centrifugate was stored at −20°C until used for enzyme assays and CsCl density gradient centrifugation.

α-Glucosidase activity was assayed by measurement of glucose liberated from maltose by a Tris-glucose oxidase-peroxidase method,4,7 and α-amylase activity, by measurement of reducing sugar produced from β-limit dextrin with Somogyi-Nelson method5,8,9 The standard assay mixture contained, in a total volume of 0.5 ml, 40 mm sodium acetate buffer (pH 5.4) for α-glucosidase, pH 6.0, containing 5 mm CaCl2 for α-amylase, 5.8 mm maltose or 2 mg/ml β-limit dextrin, and enzyme. One unit of α-glucosidase activity was defined as the amount of enzyme hydrolyzing 1 μmol of maltose/min at 37°C and that of α-amylase was defined as the amount of enzyme producing reducing sugar equivalent to 1 μmol of maltose/min at 37°C.

Figure 2 shows the changes in three starch-hydrolyzing enzyme activities during germination. No enzyme activities were found in dry seeds and 0-d seedling cotyledons. As we have reported, α-glucosidases can be isolated in active form from the rest of seeds of rice,10 buckwheat,11 sugar beet,12 sweet corn,13 and glutinous rice.14 In kidney bean, α-glucosidase activity appeared after the 2-d germination stage and increased exponentially up to about the 9-d stage, which seedlings showed the maximum activity, about 1.5 units/cotyledon. α-Amylase, which hydrolyzed β-limit dextrin to maltooligosaccharides, showed a similar increase pattern in activity. α-Amylase activity reached the maximum at about 7- to 8-d of germination (about 5.3 units/cotyledon). We also examined debranching enzyme and β-amylase activities. The former, the activity of which was measured by using pullulan as the substrate, was very low, and the latter was negligible since there was no difference in zymograms containing soluble starch or β-limit dextrin through the entire germination period (data not shown). Thus, starch granules stored in kidney beans are mainly hydrolyzed by α-amylase and α-glucosidase during germination.

α-Amylase molecules of barley,15 rice,12 and mung bean12,13 have been discussed to be synthesized de novo during development of seeds.

Fig. 1. Changes of Starch-hydrolyzing Enzyme Activities on Germinating Stages of Kidney Bean.

Fig. 2. Equilibrium Distribution in CsCl Gradients of α-Glucosidase Activity (□) and α-Amylase Activity (○) from Kidney Bean Cotyledons Grown in H2O and 80% D2O.
The density labeling technique is useful to investigate whether an enzyme is synthesized de novo or not. In the method, a compound containing a heavy isotope which can be incorporated into amino acids used for protein synthesis is supplied to the tissues. The isotope incorporation causes an increase in the density of protein separable by isopycnic equilibrium centrifugation. For this experiment, kidney bean seeds were soaked and grown in the presence of 80% D_2O (20% filtered tap water and 80% D_2O, 99.8%; Gil-sur-Yvette, France) or in H_2O. The germination in D_2O was greatly retarded, as reported by Nomura and Akazawa, due to isotopic stress of D_2O against the seeds. D_2O-germinated seeds at 11 d looked like 2-d seeds in H_2O-control, but we tested these D_2O seeds by density analysis because their activities of α-glucosidase and α-amylase were about one-tenth of those of H_2O-germinated seeds at 7 d. The procedure was essentially done by the method of Filner and Varner. The enzyme preparation, 0.7 ml, was put on 9.0 ml of 1.34 g/cm^3 density CsCl (Nacalai Tesque, Inc.) dissolved in the homogenizing buffer in a Beckman type 80 Ti rotor tube, and was centrifuged at 40,000 rpm for 65 hr at 4°C. After centrifugation, 0.2 ml of fractions were collected from top of the tube with a Density Gradient Fractionator (Instrumentation Specialties Co.). The refractive indices were measured by an Abbe refractometer (Atago Co.) at 25°C and converted to density units.

The results of the equilibrium centrifugation are shown in Fig. 2. No shift in density was observed on α-glucosidase activity. Both enzyme preparations from H_2O- and D_2O-germinated seeds showed that the density of α-glucosidase is 1.295 g/cm^3, indicating that deuterium was not incorporated into the α-glucosidase molecule and deuterium-hydrogen exchange did not occur. On the contrary, α-amylase had different densities between H_2O- and D_2O-seeds. The density of α-amylase from D_2O-germinated seeds was 1.300 g/cm^3 compared with 1.275 g/cm^3 for the control. This is a shift of 0.025 density unit or 1.96%. The density increase is similar in magnitude obtained so far by other studies: 1.9% for barley peroxidase, 1.27% for barley β-1,3-glucanase, 1.3% for barley ribonuclease, 1.5% for rice sucrose 6-phosphate synthetase, 5.5% for peanut isoamylase, and 3.5% for peanut malate synthetase. Kidney bean α-amylase is considered to be formed by using not free amino acid pools but the amino acids derived by hydrolysis of storage proteins or newly synthesized amino acids during seed germination. It seems unlikely that α-glucosidase is synthesized during germination from the pool of amino acids which are not synthesized directly by hydrolysis of storage proteins.

An activation mechanism from the pre-existing protein is suggested by the appearance of α-glucosidase activity. α-Glucosidase had been considered not to attack native starch granules, but Sun and Henson have reported that α-glucosidase from germinated barley seeds can degrade the native starch. If kidney bean α-glucosidase can also attack starch granules, it would be understandable that its activity is not observed in dry and resting seeds.

References