

**Short Communication**

**Enzymic Mechanism of Starch Breakdown in Germinating Rice Seeds**

**IV. DE NOVO SYNTHESIS OF SUCROSE 6-PHOSPHATE SYNTHETASE IN SCUTELLUM**

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Our previous work (15) demonstrated that glucose derived from reserve starch in the endosperm of germinating rice seeds as a consequence of the inducible formation of a-amylase is mobilized to the scutellum, where it is converted to sucrose. The sucrose molecules are then transported to the embryonic axis for further metabolism, e.g., the synthesis of wall polysaccharides in the developing shoot and root tissues. The enzymic assay showed that the activity of sucrose-6-P synthetase (EC 2.4.1.14) equation 1, is greater than that of sucrose synthetase (EC 2.4.1.13), equation 2,

\[
\text{UDP-glucose} + \text{fructose-6-P} \rightleftharpoons \text{sucrose-6-P} + \text{UDP} \tag{1}
\]

\[
\text{UDP-glucose} + \text{fructose} \rightleftharpoons \text{sucrose} + \text{UDP} \tag{2}
\]

\[
\text{sucrose-6-P} + \text{H}_2\text{O} \rightarrow \text{sucrose} + \text{Pi} \tag{3}
\]

during the entire germination period, although the two enzymes were not resolved successfully. Several recent investigations (5, 16) support the idea that sucrose-6-P synthetase coupled with sucrose-6-P phosphatase, equation 3, is the predominant pathway of sucrose biosynthesis in plants, whereas the principal role of sucrose synthetase is to mediate cleavage of sucrose molecules (8, 13, 16). The presence of sucrose synthesizing and cleaving systems in the germinating rice scutellum affords an ideal model to study the enzymic mechanism of sucrose metabolism in plants. In this paper we report the feasibility of utilizing the density labeling technique (9) to examine the de novo synthesis of sucrose-6-P synthetase in rice scutellum. The method has been successfully used to establish the de novo synthesis of various plant enzymes, such as a-amylase (6), protease (10), peroxidase (1), ribonuclease (3), and \(\beta\)-1,3-glucanase (3) in barley, and isocitrate and malate synthetase in germinating peanut cotyledons (7, 12).

**MATERIALS AND METHODS**

**Rice Seed Germination.** Methods of sowing and growing rice seeds (Oryza sativa L. var. Tokai Asahi) in the dark chamber at 30°C were the same as those reported previously (14, 15). However, to prepare seeds for the density labeling experiment, they were first sterilized in 0.07% Takeda-Mer solution for 6 hr and then thoroughly rinsed with \(\text{H}_2\text{O}\) before letting them imbibe for 3 days in 80% \(\text{D}_2\text{O}\) purchased from the Japan Isotope Center, Tokyo. These seeds were then germinated by placing them in a Petri dish moistened with 80% \(\text{D}_2\text{O}\). Control samples were treated with deionized \(\text{H}_2\text{O}\) throughout the experiment. The germination of seeds, in particular, root development, was greatly inhibited in 80% \(\text{D}_2\text{O}\), in agreement with previous reports by Lewis (11), Caldwell and Doebbeling (4) and Anstine et al. (1). As shown in Figure 1, \(\text{D}_2\text{O}\)-grown seeds at 14 days were more imbibed and swollen than the \(\text{H}_2\text{O}\)-seeds at the 6-day stage, but we subjected these two samples for the comparative studies. For the analysis of enzymic activities, 100 scutella dissected from each of \(\text{H}_2\text{O}\) (6-day) and \(\text{D}_2\text{O}\) (14-day) treated seeds were homogenized in an ice-chilled glass homogenizer with 1.0 ml of 0.05 M tris-acetate buffer (pH 7.0) containing 5 mm dithiothreitol, 0.1 mm EDTA, and 50 mm potassium acetate. The homogenate was centrifuged (20,000g, 20 min), and the supernatant was transferred to a small column (0.7 × 6.0 cm) of Sephadex G-25 (fine), equilibrated by the homogenizing buffer, to remove low molecular sugars. The eluate was applied to the sedimentation experiment.

**Isopycnic Centrifugation.** The equilibrium centrifugation was carried out essentially following the method described by Filner and Varner (6) and Bennett and Chrispeels (3). A 0.5-ml aliquot of the Sephadex eluate, containing the enzyme and 0.2 mg of crystalline bacterial a-amylase (Nagase Sangyo Co. Ltd., Osaka) as an internal marker, were layered over CsCl (99.9%, Gallard and Schlesinger Corp., N. Y.) dissolved in the homogenizing buffer. The final solution which had a volume of 3.5 ml and an average density of 1.35 was covered by a paraffin oil. The sedimentation was carried out in a swinging bucket rotor, SW 65 Ti, in a Spinco Model L-2 preparative type ultracentrifuge, for 65 hr at a rotor speed of 40,000 rpm to reach equilibrium at 4 C. At the end of centrifugal run, 35-μl fractions were collected, and aliquots from each were used for (a) assaying enzymic activities and (b) calculating buoyant density by measuring the refractive indices of small samples obtained across the gradient with an Abbé refractometer.

**Enzyme Assay.** Activities of sucrose-6-P synthetase and sucrose synthetase were measured by a method reported previously (2, 15) but modified as follows. The reaction mixture contained (in μmole): UDP-glucose, 0.88; fructose-6-P (or fructose), 0.5; and 10 μl of enzyme preparation in a total volume of 20 μl. The reaction was carried out at 37°C for 30 min, and the amount of sucrose-6-P or sucrose formed was determined according to the resorcinol method of Roe (17) by measuring the absorbance at 490 nm. The a-amylase activity was monitored

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RESULTS AND DISCUSSION

The patterns of equilibrium centrifugation in CsCl gradients (Fig. 2) show that sucrose-6-P synthetase, in the scutellar extract prepared from D2O-treated seeds, was distributed over a wider range of density compared to that in the extract of control seeds. The peak density of the former was 1.323 g/cm³ compared to 1.303 g/cm³ of the control sample. This 0.02 unit density shift is approximately an increase of 1.5%. This density increase was of the same magnitude observed with other enzymes (1, 3, 6), strongly indicating the de novo synthesis of sucrose-6-P synthetase in the scutellum of germinating rice seeds. However, the density shift was less than the maximum shift expected if D atoms had replaced all the C-bound H atoms in protein molecules (9). It is inferred that the source of the newly synthesized enzyme molecule is partly derived from amino acid pools and carbohydrates in endosperm tissues. This inference that amino acids resulting from the proteolytic hydrolysis of reserve protein may be utilized for the de novo enzyme synthesis in the organ is based on the electron microscope study by Swift and O'Brien (18), which demonstrated the mobilization of protein bodies in scutellar epithelium in wheat seeds shortly after the H2O imbibition. The presence of sucrose synthetase in the collected fractions was not detected positively due to its low activities in both control and D2O-treated seeds. Under the assaying conditions used, the specific activities of sucrose synthetase in both samples ranged from 0.06 to 0.1 μmole of sucrose formed/30 min-scutellum, although the enzyme activity in the D2O-treated seeds was found to be consistently higher than that in the control. These results support the current concept that the predominant pathway of sucrose formation in plants is via sucrose-6-P synthetase.

Fig. 2. Equilibrium centrifugation patterns of control (H2O-imibed) sucrose-6-P synthetase (upper) and D2O-treated sucrose-6-P synthetase (lower). The experimental details of preparing enzyme solution from H2O- and D2O-imibed rice seed scutella, CsCl gradient centrifugation, and enzyme assays are described in the text.

CONTROLLED EXPERIMENTS

Control (H2O) 80% D2O 14- days

Fig. 1. Developmental growth of H2O and 80% D2O imibed rice seeds. Left: control (H2O-imibed), 6 days; right: 80% D2O-treated, 14 days.

LITERATURE CITED


