

Enzymic Mechanism of Starch Breakdown in Germinating Rice Seeds¹ II. Scutellum as the Site of Sucrose Synthesis

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Abstract. In a close parallel to the developmental pattern of α -amylase activity, a rapid increase of maltase activity occurred in the endosperm tissue of germinating rice seeds after about 4 days of the seed imbibition. The overall pattern of the 2 hydrolytic enzyme activities strongly suggest that amyolytic breakdown is the major metabolic route of starch utilization in the germinating rice seeds. Results of the chemical analyses of sugar constituents as well as the measurements of sucrose synthetase activity show that the scutellum is the site of sucrose synthesis in the germinating rice seeds. It is thus supported that glucose derived from the reserve starch in endosperm is transported to scutellum, where it is converted to sucrose. Sucrose is further mobilized to the growing tissues, shoots and roots.

In a previous paper of this series (10), we presented experimental evidence indicating the inducible formation of α -amylase in the endosperm tissue of germinating rice seeds. Results of the chemical analyses of carbohydrate constituents in germinating seeds also support the amyolytic breakdown of reserve starch, and suggest that the phosphorolytic breakdown occupies a rather minor role. The sucrose molecule, comprising the major portion of the soluble sugar in the dry seed, was consumed for respiratory purpose in the very early stage of the germination, but no sucrose synthetase activity was detected in the endosperm tissues throughout the germination period. It was suggested that sugar transport from the endosperm to the growing embryonic tissues may be in the form of glucose or that resynthesis of sucrose may occur in the scutellum, which is the link between endosperm and embryonic axis. Chemical analyses showed that glucose comprises the greatest proportion of the soluble sugar in the endosperm in later germination stage. Another possible interpretation was that potent invertase activity interfered with the determination of the sucrose present in the tissue. The present paper deals with the experimental results showing that a marked increase of maltase activity in the endosperm tissue starts after 4 days of seed germination and that the scutellum is the site of sucrose synthesis in the germinating rice seeds.

Materials and Methods

Rice Seed Germination. Throughout the study, the same procedure of sowing rice seeds (*Oryza sativa* L. var. Fujiminori) and growing in the dark chamber as reported previously were used (10).

Enzyme Assay. Maltase was assayed by the following procedure. Endosperm tissues of 40 rice seeds devoid of roots, roots and scutella (approximately 1.3 g fresh wt) were ground with 4 ml of 0.01 M tris-HCl buffer (pH 7.5) in a chilled mortar and the whole homogenate centrifuged at 4°. An aliquot (1.5 ml) of the supernatant fraction was applied onto a column of Sephadex G-25, which was equilibrated by 0.01 M tris-HCl buffer (pH 7.5). and the eluate (4.5 ml) was used as the enzyme source. The reaction mixture contained (in μ moles): Na-acetate buffer (pH 4.5), 2.5; maltose, 0.25; and enzyme preparation, 20 μ l, in a total volume of 30 μ l. The reaction (35° for 60 min) was stopped by adding 0.5 ml of 0.1 N NaOH. The amount of glucose formed was analyzed by determining the increase of the reducing sugar content using the Somogyi-Nelson method (11). It was confirmed that crystalline bacterial α -amylase (Nagase Sangyo Company Ltd., Osaka) did not hydrolyze the maltose molecule.

In the analysis of sucrose synthetase, 100 scutella of germinating rice seeds were homogenized in a chilled small-size glass homogenizer using 0.8 ml of 0.01 M tris-HCl buffer (pH 7.5). The whole homogenate was centrifuged and a 0.5 ml aliquot of the supernatant fraction was passed through a column of Sephadex G-25 which was equilibrated by 0.01 M tris-HCl buffer (pH 7.5). The eluate (2.5 ml)

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served as the enzyme source. Reaction mixture contained (in μ moles): tris-HCl (pH 7.5), 50; $MgCl_2$, 1.25; UDP-glucose, 1.0; fructose or fructose 6-P, 1.25; Na-molybdate, 1.0; and 0.1 ml of enzyme preparation in a total volume of 0.5 ml. The reaction (35° for 30 min) was stopped by adding 0.3 ml of 2 N NaOH, and the amount of sucrose formed was determined by the resorcinol method (12).

The enzyme preparations described above were used for the measurement of invertase activity. The reaction mixture contained (in μ moles): Na-phosphate buffer (pH 6.0) 50; sucrose, 12.4; 0.5 ml enzyme solution in a total volume of 1.0 ml. The reaction was at 35° for 60 min and the amount of sucrose hydrolyzed was determined by the Somogyi-Nelson method (11).

Carbohydrate Analysis. Dowex-I ion exchange column chromatography was used to fractionate the sugar component following the method reported previously (9, 10). Scutella of 250 rice seeds at various germination stages were ground with 1 ml of 0.6 N $HClO_4$ in a chilled small-size glass homogenizer. The extraction was repeated again in the same manner. The insoluble material was washed 3 times with H_2O and 2 times more with acetone. The whole extract (about 5 ml) was neutralized with 5 N KOH to pH 6.0, and placed in the deep-freeze. Afterwards, the precipitate formed was removed by centrifugation. The supernatant fraction was diluted to 8 ml with 0.005 M $Na_2B_4O_7$. It was then applied to a column of Dowex-I anion exchange resin (borate form, 0.9×5 cm), and chromatographed by stepwise elution with 0.005 M, 0.02 M, and 0.03 M $Na_2B_4O_7$. Both sucrose and fructose were assayed by the resorcinol method (12). Maltoligosaccharides and glucose were measured by the Somogyi-Nelson method (11).

Microscopic Experiment. A median longitudinal section of developing rice seed (2 days after imbibition) was prepared after the method of Jensen (7), employing the iron-haematoxylin staining.

Results and Discussion

Results presented in Fig. 1 show a rapid increase of the maltase activity in the endosperm after about 4 days of the seed imbibition. The activity reached a maximum at around the tenth day, and declined gradually thereafter. The developmental pattern of maltase was analogous to the one of α -amylase as reported previously (10), and the overall results strengthen our view on the amyolytic breakdown of starch reserve in the germinating seed. In contrast to α -amylase, however, we observed an initial increase in maltase activity, then a loss of activity during the next 3 to 4 days followed by a notable increase of the enzyme activity. Swain and Dekker (14) have reported that the activity of maltase was detectable in the dry pea seeds. A study of Briggs (2) has shown a marked increase of maltase activity

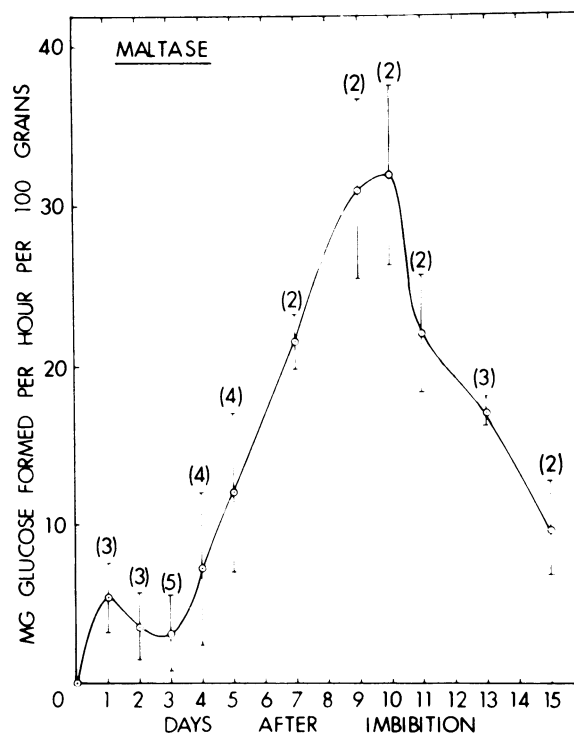


FIG. 1. Maltase activity in extracts of germinating rice seed endosperm. Details of experimental conditions are described in text. Vertical bar represents the extent of the variations of analytical results encountered. Numbers of different experiments are marked in parentheses and points are the average values.

in germinating barley endosperm under the influence of gibberellic acid, indicating the hormone-induced *de novo* enzyme synthesis. At the tenth day, it can be estimated that about 3 mg maltase are hydrolyzed per 10 grains, hydrolytic activity being much lower than the amount of starch broken down by the action of α -amylase [about 30 mg per 10 grains, *cf.* (10)]. It will be noted, however, that in the latter enzyme assay system the measurements of the unit enzyme activity were based on achromatic colorimetry and the net amount of maltose formed was not estimated.

Our earlier analytical results on the sugar constituents showed that glucose is the major soluble sugar component in the rice endosperm at later stages of germination, although sucrose comprised the major sugar in the dry seed. A question was then raised as to the form of sugar transport from endosperm to embryonic tissues. As in the case of most other plant tissues sucrose is considered to be the form of transport. We detected a slight but a gradual increase in the content of sucrose in the endosperm, but we could not detect the activity of sucrose synthetase in the endosperm throughout the germination stage. In order to examine the possibility that glucose is transported from endosperm to scutellum, where it is converted to sucrose and mobilized into embryo, we measured the sucrose

synthetase activity as well as the sugar content of the scutellum.

Microscopic section of the median longitudinal plane of germinating rice seed (2 day) clearly shows the close contact of scutellum to endosperm (Fig. 2). Often speculation has been made concerning the role of scutellum in the embryonic development of gramineae based on such plant anatomical studies. But there is also experimental evidence that the scutellum secretes digestive enzymes that hydrolyze the reserve starch in the endosperm, and eventually the scutellum translocates soluble sugars to the growing parts of embryo (4). Results shown in Fig. 3 present the



FIG. 2. Microscopic profile of developing rice seed. Magnification (X70). E: endosperm; Ep: epithelium; Sc: scutellum; C: coleoptile; R: root.

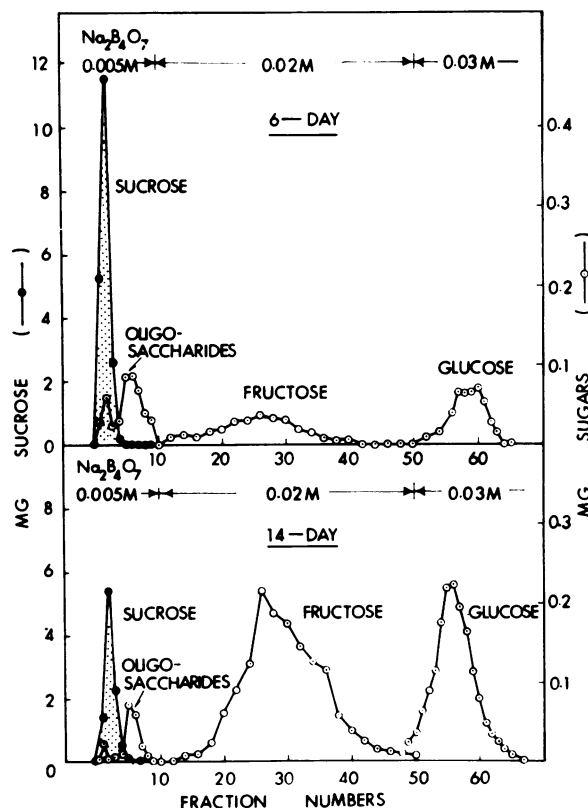


FIG. 3. Dowex-1 ion exchange column chromatography of soluble sugar fractions isolated from germinating rice seed scutella. Details of experimental procedures are described in text.

data of Dowex-I ion exchange column chromatography of the soluble sugar component of the scutellum, and results of the time-sequence analyses are shown in table I. They clearly show that sucrose accounts for the greatest portion of the soluble sugar in the scutellum, while maltooligosaccharides, fructose, and glucose are minor components. Due to the technical difficulties, we could not determine the amount of sugar constituents in the scutellum at the very early germination stage. Such a pattern of sugar distribution in the scutellum is sharply contrasted to the one in endosperm tissues [cf. Fig. 2 of (10)]. It will be noted that until the eleventh

Table I. Content of Sugars in Scutellum of Germinating Rice Seeds

Time after imbibition <i>days</i>	Sugars			
	Sucrose	Maltooligosaccharides (as maltose)	Glucose	Fructose
4	8.3	0.01	0.34	0.50
6	7.9	0.19	0.16	0.25
8	7.8	0.22	0.34	0.55
11	7.4	0.08	0.47	0.51
14	4.1	0.08	0.69	1.17

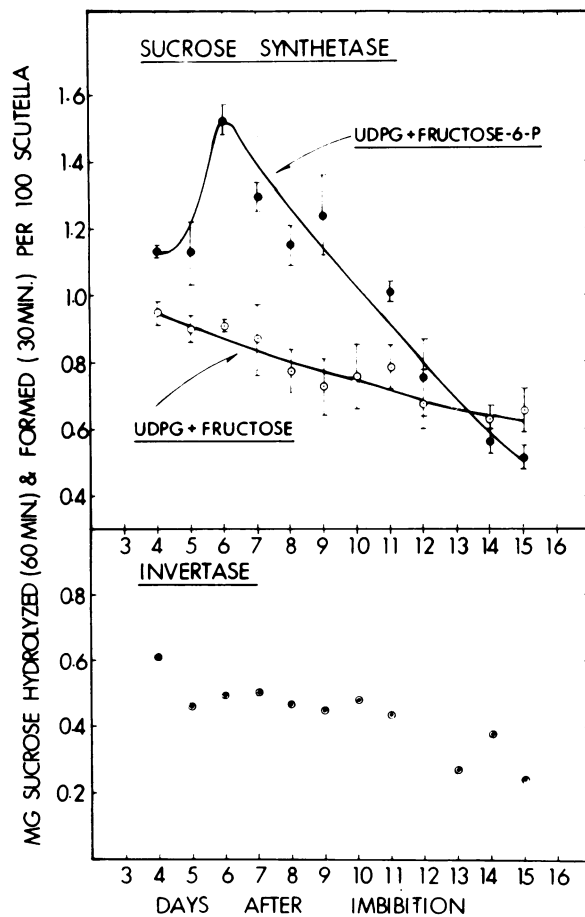


FIG. 4. Sucrose synthetase and invertase activity in extracts of germinating rice seed scutella. Sucrose synthetase: Methods of experimental conditions are described in text. The use of either fructose or fructose 6-P as the glucose accepting molecule is marked as the respective symbols in the Fig. Duplicate sets of experiments in the 2 sequential analyses are marked by the bars showing the variations of analytical results. Invertase: Each circle represents the results of a single analysis using extracts of germinating rice seed scutella. Details of experimental conditions are described in text.

day, the sucrose content remained roughly constant, afterwards it declined rather abruptly (table I).

The activity of sucrose synthetase in extracts of scutellum taken from seeds at different germination stages was measured employing fructose and fructose 6-phosphate as the glucose-accepting molecules (Fig. 4). It can be seen that the enzyme activity of the scutellum was potent from the early germination stage, the unit enzyme activity (mg sucrose formed per single organ) being much superior to that in shoots or roots [cf. table I of (10)]. Two notable observations emerge from the results. One, it can be seen that the decline of the enzyme activity occurring after the eleventh day was more marked than that in the preceding period. Thus, comparing

the enzyme activity with the results of sucrose analysis (table I), it is interesting that the rate of the sucrose synthesis in the scutellum appears to parallel the rate of movement of sucrose from scutellum to the embryonic axis, assuming the *in vitro* enzyme assay reflects the synthesis of sucrose *in vivo*. Two, the enzymic synthesis of sucrose-P appears to be more dominant than sucrose synthesis in the early germination stage. Nobody has succeeded in resolving 2 different enzymes distinguishing between the synthesis of sucrose and sucrose-P (1,3), although a study of Slabnik *et al.* (13) has indicated that there are some different properties between the 2 enzyme reactions. We have not examined the problem further, and it remains obscure whether or not sucrose-P is synthesized predominantly in the scutellum of germinating rice seeds. Results further support our previous implication (10) that a transient increase of the sucrose content in endosperm after about 4 days of the germination may be due to the possible "backflow" of sucrose from embryonic tissue to the endosperm.

Simultaneous measurement of the invertase activity in the scutellum shown in the lower part of Fig. 4, indicates that the hydrolytic breakdown of sucrose is not particularly potent in the tissue as compared to its synthesis throughout the germination stage.

Edelman *et al.* (5) reported that the set of enzymes necessary for the synthesis of sucrose from glucose, *i.e.*, hexokinase, phosphoglucosomerase, phosphoglucomutase, UDP-glucose pyrophosphorylase, sucrose synthetase and UDP-ATP-kinase, occur in the scutellum of germinating wheat and barley seeds. But they have not conducted the time-sequence analyses of the enzyme as well as the sugar contents in the tissue. Thus our present findings, basically in agreement with their report, strengthen the notion that glucose derived from the endosperm by amylolytic breakdown is mobilized in the scutellum, where it is resynthesized to sucrose. The latter is then transported to the embryonic axis, *i.e.*, shoots and roots, for further metabolic purposes.

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