ENZYMIC MECHANISM OF STARCH SYNTHESIS IN GLUTINOUS RICE GRAINS [1]

Takao Murata, Tatsuo Sugiyama, and T. Akazawa

The International Rice Research Institute [2]

Los Baños, Laguna, The Philippines

Received December 7, 1964

Although a considerable amount of evidence has been accumulated showing the important role of ADPG in the biosynthesis of starch in various plant organs (De Fekete and Cardini, 1964; Murata et al., 1964 b), the enzymic mechanism of starch formation in the grains of glutinous (waxy) varieties of the cereal grains has remained obscure. Nelson and Rines (1962) reported the absence of UDPG-starch transglucoysiase in starch granules prepared from waxy maize seeds, and proposed a different pathway of amylopectin biosynthesis from that of amylose. Recently, Nelson and Tsai (1964) obtained a piece of evidence that ADPG-starch transglucosylase in waxy maize is localized in the starch granules derived from embryo and maternal tissues, but not in the endosperm, which is the principal site of starch synthesis in the seed. They found that the transglucosylase activity of waxy maize is about one tenth of the magnitude of that of non-waxy maize and essentially all of its activity can be accounted for by the starch granules present in the sporophytic tissues. The lower transglucosylase activity of waxy maize has been observed by Frydman (1963).

By using six different glutinous rice varieties, we have found a complete absence of ADPG (UDPG)-starch transglucosylase in the starch gra-

---

[1] Issued as IRRI Journal Series No. 29

[2] Mailing address: IRRI, Manila Hotel, Manila, The Philippines.

nules obtained from the developing grains. The typical results are shown in Fig. 1. Furthermore, we have been unsuccessful in demonstrating enzyme activity even by employing several other experimental techniques for getting starch granules.

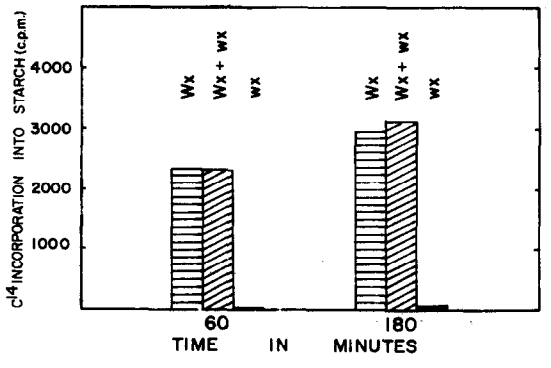

Fig. 1. ADPG-starch transglucosylase activity of non-glutinous (Wx) and glutinous (wx) rice starch granules.

Acetone powder starch granules were prepared after the method of Akazawa et al., (1964), and the assay method as reported by Murata et al., (1964 b). The reaction mixture contained glycine buffer (pH 8.4), 40 μmoles; EDTA, 0.1 μmole; ADP-glucose-C^{14}, 0.34 μmole (4,200 c.p.m.); and starch granules 2.0 mg, except in the case of (Wx + wx), 2.0 mg of each kind of starch.

However, from the essentially identical patterns of the nucleotides in non-glutinous and glutinous rice grains, as portrayed in Fig. 2, it can be surmised that a similar enzymic mechanism operates for starch synthesis in these two different genetic stocks. In fact, by feeding sucrose-C^{14} to developing rice panicles at the mid-milky stage, glucose was found to be readily incorporated into the starch molecule at approximately the same speed in the case of both non-glutinous and glutinous grains (Murata and Akazawa, in prepn.). Subsequently, we explored the localization of ADPG-starch transglucosylase activity in various fractions of glutinous rice grains, and found that the activity is almost exclusively present in the supernatant of the 100,000 x g centrifugation.

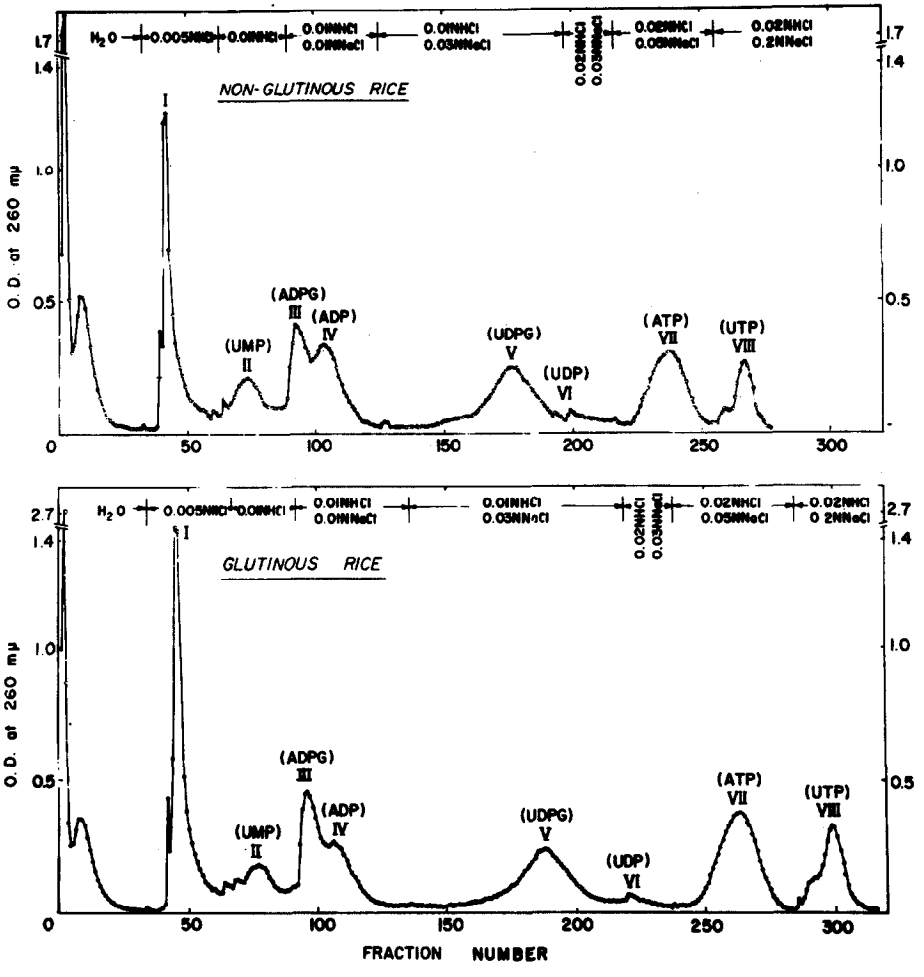

Fig. 2. The distribution of nucleotides in developing grains of non-glutinous and glutinous rice.

In each case, about 40 g of the freshly harvested grains at the mid-milky stage were treated in the same way as that reported by Murata et al., (1964 a), and the individual nucleotides were separated by a Dowex-I anion exchange column chromatography accordingly.

About 2-3 g of glutinous rice grains (variety Pah Leuad of the indica type) at the mid-milky stage were ground in a small mortar at 0°C; the entire grain contents were collected, and suspended in 2.0 ml of 0.07 M phosphate buffer (pH 7.5) containing 0.035 M glutathione and 0.07 M EDTA which we call the whole grain suspension. It was then spun in a refrige-

rated centrifuge at 6,400 x g for 5 minutes to separate 1.7 ml of liquid, which is referred to as supernatant I. This liquid was centrifuged in a Spinco preparative ultracentrifuge at 100,000 x g for 1 hour to get 1.5 ml of liquid (supernatant II). The precipitate was then suspended in 1.4 ml of the same media as used above for the whole grain suspension. As shown in

Table I

The distribution of ADPG-starch transglucosylase in various fractions of glutinous rice grains

Enzyme source	C^{14} incorporation into starch	
	Total c.p.m.	%
Whole grain suspension	2,170	36.8
Supernatant I	1,360	23.1
Supernatant II	1,220	20.7
Boiled supernatant II (100°C, 5 min.)	123	2.1
100,000 x g precipitate	179	3.0

Basic composition of the reaction mixtures are: glycine buffer (pH 8.4), 4 µmoles; glucose-6-phosphate, 0.5 µmole; NaF, 3 µmoles; ADP-glucose-C^{14}, 0.219 µmole (5,900 c.p.m.); and enzyme preparation in a total volume of 31 µl. Whole grain suspension (20 µl) containing inorganic phosphate, (1.4 µmoles), EDTA (1.4 µmoles), and glutathione (0.7 µmole) consists of 3-3.5 mg of starch. In each of the remaining reaction systems, in addition to 3 mg of acetone powder starch granules of the same origin, 20 µl of the individual preparation was added. Incubation was at 37°C for 60 minutes.

Table I, about 37% of the glucose was transferred from ADP-glucose-C^{14} to the starch molecule in the whole grain suspension, which was at a rate of approximately one half as that in the corresponding preparation of non-glutinous rice grains. The transglucosylase activity remained essentially in the soluble fraction which was not sedimental by repetitive centrifugation, and no enzymic activity was detectable in the final precipitate. It seemed evident that phosphorylase was not involved in the overall process because of the very low magnitude (less than 1%) of glucose transferred from glucose-1-phosphate-C^{14}. Fig. 3 shows that starch granules devoid of

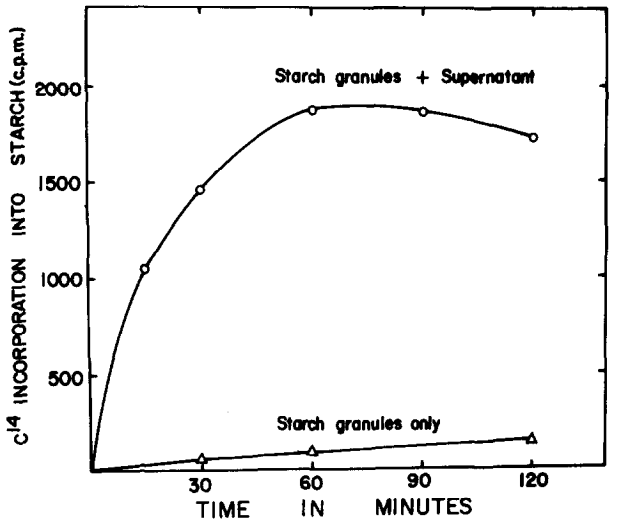

Fig. 3. ADPG-starch transglucosylase activity in glutinous rice.

Acetone powder starch granules were prepared in the same way as in the experiment shown in Fig. 1, whereas supernatant II, as described in the text, served as the soluble enzyme source. Reaction mixture for the starch granules only contained glycine buffer (pH 8.4), 4 μmoles; EDTA, 0.2 μmole; glutathione, 0.5 μmole; glucose-6-phosphate, 0.5 μmole; ADP-glucose-C^{14}, 0.219 μmole (5,900 c.p.m.); and starch granules, 3.0 mg in a total volume of 15 μl. In a complete reaction system containing the supernatant II, the composition was the same as that described in Table I. Incubation was at 37°C.

ADPG-starch transglucosylase activity served as an efficient glucose acceptor when mixed with the non-dialyzed supernatant II. Actually, about 32% of the glucose was transferred. The slight decline in the amount of glucose transferred under prolonged incubation appears to be attributable to the breakdown of starch molecules by the reversal of the phosphorylase reaction which proceeds favorably under the experimental system employed, and also to a certain amount of sucrose formed from ADPG.

As a result of our studies the following additional statements can be made with respect to the soluble ADPG-starch transglucosylase reaction in glutinous rice grains: (a) dialysis does not affect the enzymic activity; (b) glucose-6-phosphate has no stimulating effect on the reaction; (c) monoiodoacetamide (2.5×10^{-3} M) shows an almost complete inhibition; (d) maltose

is a major radioactive β-amylolysis product of the enzymically synthesized starch molecule, which indicates the formation of α(1→4) glucosidic linkages in the enzymic process; and (e) non-glutinous rice starch, corn starch, amylose, amylopectin, and glycogen are equally active as glucose acceptors.

From the initial discovery by Leloir and his associates (1961), starch synthethase of various sources involving UDPG and ADPG as glucose donors has been reported to be commonly a particulate enzyme firmly adhered to starch granules and resistant to solubilization. The present study indicates for the first time that in developing glutinous rice grains, ADPG-starch transglucosylase exists in a soluble form, though the mode of enzyme action appears to be the same as that of the particle-bound enzyme. Thus, it is expected that future kinetic studies on the starch synthesizing mechanism will be greatly facilitated by being able to use the enzyme in its soluble form. Starch in glutinous cereals consists essentially of highly branched amylopectin molecules, and whether or not the genetic control of the transglucosylase distribution in the plant cell is a determinant of their biosynthesis awaits further elucidation. It is interesting to note in this connection that the enzymes engaged in the biosynthesis of some other branched glucans, i.e. glycogen (Rosell-Perez and Larner, 1964), phytoglycogen (Frydman and Cardini, 1964) and bacterial glycogen (Shen et al., 1964) have been isolated in a soluble form.

REFERENCES

Akazawa, T., Minamikawa, T., and Murata, T., Plant Physiol. 39, 371 (1964).
De Fekete, M.A.R., and Cardini, C.E., Arch. Biochem. Biophys. 104, 173 (1964).
Frydman, R.B., Arch. Biochem. Biophys. 102, 242 (1963).
Frydman, R.B., and Cardini, C.E., Biochem. Biophys. Res. Comm. 14, 353 (1964).
Leloir, L.F., De Fekete, M.A.R., and Cardini, C.E., J. Biol. Chem. 236, 636 (1961).
Murata, T., Minamikawa, T., Akazawa, T., and Sugiyama, T., Arch. Biochem. Biophys. 106, 371 (1964 a).
Murata, T., Sugiyama, T., and Akazawa, T., Arch. Biochem. Biophys. 107, 92 (1964 b).
Nelson, O.E., and Rines, H.W., Biochem. Biophs. Res. Comm. 9, 297 (1962).
Nelson, O.E., and Tsai, C.Y., Science 145, 1194 (1964).
Rosell-Perez, M., and Larner, J., Biochemistry 3, 75, 81, 773 (1964).
Shen, L., Ghosh, H.P., Greenberg, E., and Preiss, J., Biochim. Biophys. Acta 89, 370 (1964).