THE ROLE OF ADENOSINE DIPHOSPHATE GLUCOSE IN LEAF STARCH FORMATION

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Starch resulting from photosynthetic carbon assimilation accumulates in the chloroplasts of the leaf tissues of many higher plants. In contrast to the reserve starch which is stored in organs such as tubers, roots, grains and leaf sheaths, the starch accumulating in the chloroplasts is known as "assimilation starch" and is considered to be a more transient form, being consumed readily as metabolic fuel. It has been assumed that leaf starch is synthesized by the phosphorylase reaction, but the presence of phosphorylase in chloroplasts has been a matter of dispute for many years (Yin, 1948; Stocking, 1952; Porter, 1953; Madison, 1956), although non-aqueous extraction procedures have shown that about 50% of the total phosphorylase is located in the chloroplasts (Stocking, private communication). In the light of the recent finding on the role of ADPG-starch transglucosylase in starch synthesis (Frydman, 1963; De Fekete and Cardini, 1964; Murata et al., 1963, 1964), we have attempted to examine this alternate pathway in the formation of leaf starch. Studies on the role of ADPG have so far been confined to the enzymic synthesis of reserve starch, but the present experimental results provide evidence that the same mechanism operates in the chloroplasts.

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The presence of starch granules in chloroplasts has been clearly revealed by electron microscopic studies of several plants, e.g., spinach, tobacco, and beans (Weier et al., 1963), and we thought of the possibility that such granules per se are the site of the enzymic synthesis of leaf starch. Starch granules were prepared from 170 g of leaf blade tissue of the soy bean (Phaseolus aureus) freshly harvested at 1.00 p.m. in the field, following the method as reported previously (Akazawa et al., 1964). The tissue was ground with 500 ml of chilled water in a cold room (0°C), and strained twice through 4 layers of cheese cloth. The extract was centrifuged at 350 x g for 5 minutes, and the precipitate was washed once with 500 ml and again with 200 ml of water. To remove the slightly contaminated cell debris from the starch granules, the total precipitate was suspended in 200 ml of chilled water and centrifuged at 20 x g for 1-2 minutes; this procedure was repeated twice. The supernatant was centrifuged again to get the starch granules, which were washed three more times with chilled acetone (-15°C). The resulting white granular sample was dried in vacuo over P_2O_5. The total nitrogen content was determined to be 4.43 μg per mg of starch granules.

The assay of the starch synthetase activity of the granules was carried out essentially in the same way as previously reported (Akazawa et al., 1964; Murata et al., 1964); after incubation with ADP-glucose-C^{14} and UDP-glucose-C^{14} for chosen reaction intervals at 37°C, the magnitude of the transfer of the glucose-C^{14} unit to the starch molecule was analysed with a liquid scintillation spectrometer.

As shown in Fig. 1, the glucosyl group was transferred very rapidly from ADPG to the starch molecule, reaching a maximum level after 30 minutes of incubation, whereas UDPG was entirely inactive. By increasing the amount of starch granules added, the rate of ADPG utilization increased correspondingly as shown in Fig. 2. Again UDPG was entirely inactive. The unit enzymic activity (mμmoles ADPG utilized/mg of starch granules) shows that the transglucosylation reaction proceeds far more effectively

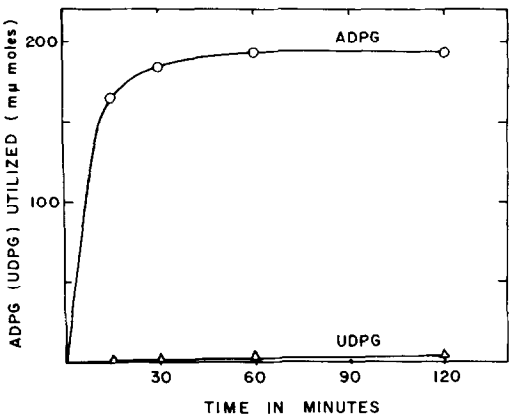

Fig. 1 Glucose transfer from ADPG and UDPG into the starch molecule by the soy bean leaf enzyme.

The compositions of the reaction mixtures were as follows (in μmoles): glycine buffer (pH 8.4), 4.0; EDTA, 0.2; ADP-glucose-C^{14}, 0.215 (3,770 c.p.m.) or UDP-glucose-C^{14}, 0.240 (15,000 c.p.m.); and 2.0 mg of starch granules in a total volume of 17 μl. Incubation was at 37°C, and the radioactivity determination of starch molecules was carried out in exactly the same way as that reported previously (Murata et al., 1964).

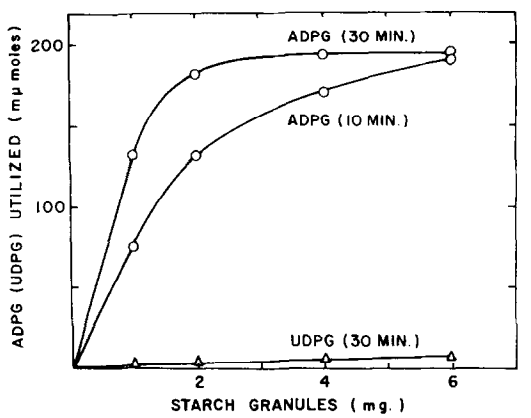

Fig. 2 Relationship between the rate of ADPG (UDPG)-transglucosylation reaction and the amount of starch granules.

Experimental conditions were exactly the same as those in Fig. 1.

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using leaf starch enzyme as compared with rice grain enzyme (cf. Murata et al., 1964). In order to confirm the formation of α(1→4) glucosidic linkage by the transglucosylation reaction, starch granules were hydrolysed by β-amylase after incubation with ADP-glucose-C^{14} and the radioactivity

distribution was determined. As shown in Fig. 3, it was present exclusively in the maltose portion.

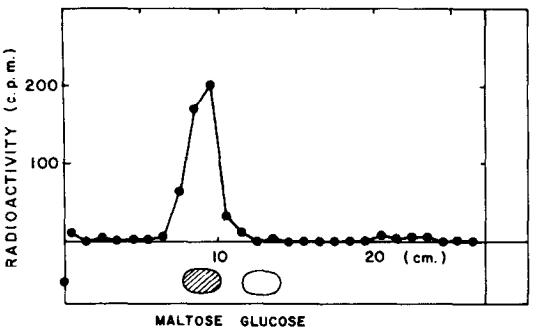

Fig. 3 Paper chromatography of the β-amylolysis product of starch granules. Starch granules (4.0 mg) incubated with ADP-glucose-C^{14} under the same conditions as shown in Fig. 1 (37°C, 60 minutes) were thoroughly washed with CH_3OH, and hydrolyzed with β-amylase (Wallerstein) for 20 hours at 37°C, following the method of Peat et al., (1952). The reaction product was treated with Amberlite MB-3 for deionization. The extract was further treated with 3 times its volume of CH_3OH, and concentrated after adjusting the pH to 7.0. One half of the total residue was applied to a sheet of Whatman No. 1 paper and developed in pyridine-ethylacetate-water (50:120:40), as a solvent. The radioactivity of the paper strips was determined using a Packard Tricarb liquid scintillation spectrometer.

The results presented in Table I show that a slight incorporation of glucose into the starch molecule occurs from glucose-1-phosphate-C^{14} alone. This fact may be ascribed to the phosphorylase reaction, but the further addition of ATP did increase the glucosyl transfer about two fold. However, UTP was inert, which indicates that ADPG-pyrophosphorylase is attached to the starch granules. This is in agreement with our previous observations using rice grains (Murata et al., 1964). There is a possibility that chloroplast starch granules are also the site of pyrophosphorylase which is intimately connected with the starch-synthetase reaction, and

Table I

Glucose Transfer from Glucose-1-Phosphate-C^{14} into the Starch Molecule

Nucleotide added (μmole)	C^{14} incorporation into starch	
	c.p.m.	%
None	1,260	1.6
ATP (0.5)	2,690	3.5
UTP (0.5)	1,140	1.5

The compositions of the reaction mixtures were as follows (in μmoles): glycine buffer (pH 8.4), 4.0; glucose-1-phosphate-C^{14}, 0.3 (7.75×10^4 c.p.m.); $MgSO_4$, 1.0; EDTA, 0.1; NaF, 2.0; ATP or UTP, 0.5; and 6.0 mg of starch granules in a total volume of 16 μl. Incubation was at 37°C for 60 minutes. Methods of radioactivity determination were the same as those in Fig. 1.

that the former was either leached out or inactivated during the process of isolating the enzyme.

The results of this experiment bear dual importance in starch biochemistry. First, they demonstrate the metabolically active nature of the starch granules in the chloroplasts, and secondly, they verify the common role of ADPG-starch transglucosylase in starch formation in plant cells in general. It has been well established by many workers that the photosynthetically produced ATP in grana (light reaction) is utilized for the CO_2 fixation leading to the formation of the hexose phosphates (dark reaction) in stroma (see Arnon, 1958), and it can be envisaged now that in the chloroplasts of many plants, ATP also directly participates in starch formation through ADPG synthesis and the subsequent transglucosylation reaction.

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