ADENOSINE DIPHOSPHATE GLUCOSE IN RICE AND ITS ROLE IN STARCH SYNTHESIS

T. Murata, T. Minamikawa, and T. Akazawa

The International Rice Research Institute, Los Baños, The Philippines

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From the classical work of Leloir and his group (Leloir, 1961), it appears very likely that starch and similar glycans are synthesized via the nucleotide sugars. Although Recondo and Leloir (1961) had found that synthetic ADP glucose was the most efficient intermediate for starch synthesis in the bean, this nucleotide sugar was not known to occur in higher plants. We have now found that ADP glucose is a natural constituent of ripening rice grains and is converted more efficiently than UDP glucose to starch by a rice enzyme. Recondo et al. (1963) have reported independently similar findings in maize.

ADP glucose was separated by Dowex-1 anion exchange column chromatography (Mori et al., 1960) of a perchloric acid extract of 1.2 kg of ripening rice grains at the mid-milky stage (Fraction V in Fig. 1). The criteria for identification of this fraction as ADP glucose were as follows:

(a) UV absorption spectra at pH 2.0 (λ_{max} = 257mμ; λ_{min} = 230mμ; A_{250}/A_{260} = 0.87; A_{280}/A_{260} = 0.23) and pH 7.0 (λ_{max} = 259mμ; λ_{min} = 228mμ; A_{250}/A_{260} = 0.82; A_{280}/A_{260} = 0.17) are almost identical with those of ADP (Beaven et al., 1955; Pabst Technical Circular, 1961).

(b) The ratios of total phosphorus, labile phosphorus, pentose, and reducing sugar to base deviated from 0.5% to 9% from theoretical (Table I). Parallel analyses were performed on UDP glucose contained in fraction VII.

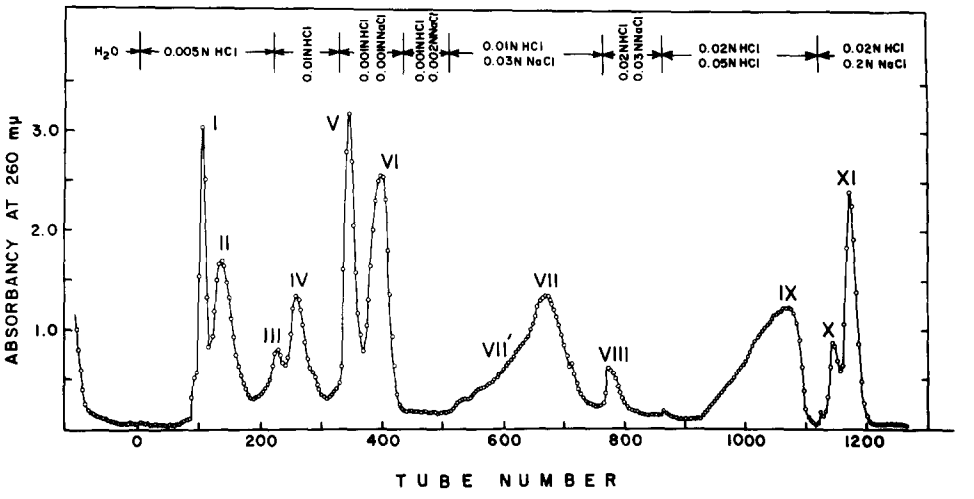

Fig. 1. Column chromatographic separation of nucleotides of developing rice grains.

A perchloric acid extract of 1.2 kg of developing rice grains was analyzed according to Mori et al. (1960). Fractions V and VII were identified as ADP glucose and UDP glucose, respectively (see text). Details of procedures and the identities of other fractions will be reported elsewhere (Akazawa et al.).

(c) Paper chromatography of the acid hydrolysate revealed a single sugar component which co-chromatographed with glucose in three solvent systems (ethyl acetate: acetic acid: water, 3:1:3; 80% w/v phenol in water; and n-butanol:acetic acid:water, 4:1:5).

(d) Upon mild acid hydrolysis (0.01 N HCl at 100° C), 99% of the total sugar was liberated within 5 minutes; this behavior was consistent with a sugar linkage at the first carbon.

(e) ADP was the only UV absorbing component after 15 minutes, mild acid hydrolysis (0.01 N HCl at 100°) (Paladini & Leloir, 1952); after 1 hour, the major product was AMP.

(f) By paper electrophoresis (Crestfield & Allen, 1955) of the alkaline hydrolysate (Paladini and Leloir, 1952) both the "fast ester", glucose-1,2-monophosphate, and the "slow ester", glucose-2-phosphate, were identified. The hydrolysate also contained AMP.

(g) Finally, fraction V and authentic ADP glucose migrated as a single component in the solvent system of Paladini & Leloir (1952) (Table I).

Table 1. Analytical data of ADP glucose and UDP glucose isolated from rice grains[a]

Fraction No.	Tube No.	Spectral type	Composition (moles per mole base)[b]				R_{AMP} values[f]				Compound identified
			Total P[c]	Labile P[c]	Pentose[d]	Reducing sugar after hydrolysis[e]	pH 3.8 a	pH 3.8 b	pH 7.5 a	pH 7.5 b	
V	334-360	Adenosine	2.01	0.93	1.09	0.98	0.72 (0.72)	0.69 (0.68)	1.30 (1.30)	1.24 (1.26)	ADP glucose
VII	640-694	Uridine	1.93	1.02	0.34	0.95	0.85 (0.85)		1.63 (1.65)		UDP glucose

a. Identification of other nucleotides in each fraction will be reported elsewhere.

b. Calculated from molar absorbancy of adenosine and uridine.

c. Method of Nakamura (1951)

d. Method of Mejbaum (1939); ADP used as standard

e. Method of Somogyi (1951)

f. Solvent system was that of Paladini and Leloir (1952); R_{AMP} values of the authentic ADP glucose and UDP glucose are shown in parentheses.

On the basis of these analyses, we concluded that fraction V was in fact ADP glucose.

Synthesis of starch from ADP glucose was effected by an enzyme from acetone powders of rice starch granules. (1.0mg of enzyme was incubated with 240 mμmoles of each nucleotide sugar under the conditions described by Akazawa et al., 1963.) A comparison of the rate of nucleotide diphosphate liberation from ADP glucose and UDP glucose is presented in Fig. 2. ADP glucose was transferred about three times as fast as UDP glucose, but levelled off after 3 hours incubation and tended to about the same final value as that of UDP glucose. In the case of UDP glucose, evidence that the sugar is in fact transferred to starch by a rice enzyme is reported elsewhere (Akazawa et al., 1963). A similar radioisotopic experiment is required to demonstrate the crucial role of ADP glucose in starch synthesis in rice.

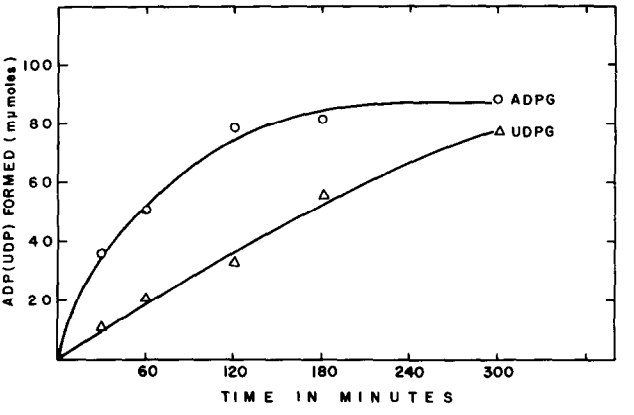

Fig. 2. Enzymatic synthesis of starch by ADP glucose- or UDP glucose-starch transglucosylase of rice.
1.0 mg of enzyme was incubated with 240 mμmoles of ADP glucose or UDP glucose; 4 μmoles glycine buffer, pH 8.4; and 2 μmoles EDTA in a final volume of 15 ml. Liberation of ADP (or UDP) was assayed by the enzymatic method (Akazawa et al. 1963).

The enzymological and chemical evidence establishes the possibility of two alternative intermediates in starch synthesis: ADP glucose and UDP glucose. We have no evidence on the pathway of starch synthesis

in vivo; but we may speculate on the possibility of some compartmentation existing in the developing rice grain controlling the synthesis and biological roles of these nucleotide sugars. The regulating mechanism for transglucosylation in carbohydrate metabolism via ADP glucose or UDP glucose systems presents an interesting problem in cellular control.

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