Solubilization of UDP-glucose-specific starch synthetase in sweet-potato (*Ipomoea batatas*) starch granules by the urea/pullulanase method

Takashi SASAKI and Keiji KAINUMA

National Food Research Institute, P.O. Box 11, Yatabe, Tsukuba, Ibaragi, Japan 305

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The ADP-glucose- or UDP-glucose-specific starch synthetase bound to sweet-potato (*Ipomoea batatas*) starch granules is localized in the granules, and the UDP-glucose-specific enzyme was solubilized by urea/pullulanase treatment of the starch granules.

Starch synthetase can be found in a soluble state and in a particulate form bound to starch granules. The soluble enzyme, which has been isolated from maize (*Zea mays*), potatoes (*Solanum tuberosum*) and other plant tissues, transfers glucose specifically from ADP-glucose (Leloir *et al.*, 1960; Cardini & Frydman, 1966; Murata & Akazawa, 1966). On the other hand, starch synthetase bound to starch granules from wrinkled pea (*Pisum sativum*), potatoes and waxy maize (*Zea mays*) can use ADP-glucose, UDP-glucose and other sugar nucleotides as glucosyl donors, but have different specificities toward the sugar nucleotides (Frydman, 1963; Frydman & Cardini, 1965; Katsura & Nelson, 1966).

In order to study the physical and enzymic properties of the bound starch synthetase and the mechanism of starch-granule formation, it is necessary to solubilize the enzyme.

A number of experiments were carried out in order to liberate the enzyme from starch granules. Detergent, dilute-alkali, proteolytic-enzyme, urea, amylase or grinding treatments of starch granules were ineffective (Chandorkar & Badenhuisen, 1966, 1967; Frydman & Cardini, 1967; Tanaka *et al.*, 1967). However, on treatment with 7M-urea, potato starch granules swelled rapidly and became separated into two parts: the contents solubilized by urea stained blue with iodine, and when these granules were washed with water, these were lost, leaving the insoluble granular bags. The products of enzyme activity remained bound to the insoluble part of the granule that was left after the urea treatment, where they were found as glucose or maltose units, probably linked to protein primer (Frydman & Cardini, 1967; Chandorkar & Badenhuisen, 1967; Tandecarz & Cardini, 1979).

On the other hand, it has been reported that 75% (v/v) dimethyl sulfoxide solubilized the bound synthetase of ripening grains of rice (*Oryza sativa*)

at the 'mid-milky' stage, and a (starch synthetase)-amylose complex was isolated that had dual specificity toward UDP-glucose or ADP-glucose (Perdon *et al.*, 1975). However, it is difficult to apply this procedure to late-stage cereal-grain starch and to starch from other tissues. The structure of starch granules may be shown to consist of inner, middle and surface layers by urea/pullulanase treatment (Sasaki *et al.*, 1980), and it is desirable to solubilize and fractionate the enzyme from each layer.

The present paper reports the solubilization of starch synthetase by urea and pullulanase.

Materials and methods

Sweet potatoes (*Ipomoea batatas*, var. *Kintoki*) were obtained from a local market. About 100g of tubers were peeled and homogenized in a juicer. The crude extract was strained through gauze and 0.2ml of 2-mercaptoethanol was added to 50ml of filtrate. The filtrate was centrifuged at 500g for 5min and the starch fraction was washed with 0.2M-phosphate buffer, pH7.3, five times until the yellow colour of starch disappeared. The starch was suspended in 4vol. of cold (−15°C) acetone and centrifuged. After the starch had been washed three times with cold acetone, it was spread out on a Petri dish and dried in a cold-room.

Urea treatment of starch granules was performed by the modified method of Cardini & Frydman (1966). The urea was dissolved in 0.1M-glycine/NaOH buffer, pH8.2. Starch granules (10mg) were treated with 1.0ml of the same buffer containing 7M-urea for 20min at 20°C and then washed five times with the same buffer without urea; the insoluble granules are referred to as the 'urea-treated starch'. The urea-treated starch was suspended in 100μl of 0.2M-glycine/NaOH buffer, pH8.2, and treated with 5μl (2 units) of pullulanase at 37°C for 20min; this insoluble starch is referred to as the

'urea/pullulanase-treated starch'. After centrifugation at 5000 *g* for 10 min the soluble and insoluble fractions were obtained.

The starch synthetase activity was assayed by the modified Cardini & Frydman (1966) method. The starch granules (10 mg), urea-treated starch (approx. 9.1 mg) or urea/pullulanase-treated starch (approx. 3.4 mg) were incubated with 100 μ l of 0.2 M-glycine/NaOH buffer, pH 8.2, and 0.015 μ Ci of ADP-[14 C]glucose (or UDP-[14 C]glucose) at 37°C for 2 h with shaking.

The reaction was stopped by adding 5 ml of 80% (v/v) ethanol, then the sample was boiled for 10 min and washed. This procedure was repeated three times until free 14 C-labelled sugar nucleotide was removed. The washed precipitate was suspended in 10 ml of Aquasol 2 and counted for radioactivity in a liquid-scintillation spectrometer.

The standard assay solution for the soluble fraction contained: 10 μ l of 0.2 M-glycine/NaOH buffer, pH 8.2, 20 μ l (500 μ g) of glycogen, 1.0 mol of NaF, 0.015 μ Ci of ADP-[14 C]glucose (or UDP-[14 C]glucose) and 30 μ l (1 mg of protein) of soluble fraction after urea/pullulanase treatment. After incubation at 37°C for 2 h, the reaction was stopped by adding 1 ml of 80% ethanol. The tube was centrifuged, the precipitate was washed by the method described above in order to eliminate traces of [14 C]sugar nucleotide, and was counted for radioactivity.

Pullulanase (40 units/mg) was obtained from Hayashibara Co., Shimoiwai, Okayama, Japan. ADP-[14 C]glucose (sp. radioactivity 271 mCi/mmol) and UDP-[14 C]glucose (sp. radioactivity 321 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. and Aquasol 2 scintillator was obtained from New England Nuclear Corp., Boston, MA, U.S.A.

Results and discussion

Starch synthetase bound to starch granules of sweet potato could utilize ADP-glucose and UDP-

glucose as glucosyl donor. Starch granules swelled rapidly to two to three times their normal size on treatment with 7 M-urea, and the inner layer (approx. 9%) could be removed by washing. A stimulation (about 3.5-fold) of [14 C]glucose incorporation from ADP-[14 C]glucose or UDP-[14 C]glucose into 7 M-urea-treated starch (urea-insoluble granules and outer layer morphologically) was observed, when compared with untreated starch.

In determining the fate of the [14 C]glucose incorporated from radioactive sugar nucleotides into intact starch granules, we found the radioactivity from ADP-[14 C]glucose mainly in the outer layer of the granules, which was insoluble in urea. On the other hand, about one-third of the radioactivity from UDP-[14 C]glucose was incorporated into the inner layer (the urea-soluble fraction) and about two-thirds into the outer layer. This suggests that the bound starch synthetase activities with specificities towards ADP-glucose or UDP-glucose are different and localized in the starch granule.

In order to solubilize starch synthetase bound to 7 M-urea-treated starch, pullulanase treatment was tried. It is known that pullulanase cleaves α 1 $\rightarrow$ 6 linkages specifically and not α 1 $\rightarrow$ 4 linkages. Accordingly the starch denatured by urea might be solubilized easily by pullulanase, and α 1 $\rightarrow$ 4-linked glucose polymers like dextrin and amylose might be formed. By the pullulanase treatment of 7 M urea-treated starch, about 57% of starch (which might be considered a middle layer morphologically) was solubilized, and about 34% of insoluble starch (a surface layer morphologically) was obtained (Sasaki *et al.*, 1980). The incorporation of radioactivity decreased after pullulanase treatment, but after urea treatment about 20% of the activity towards ADP-glucose was retained as was about 40% of that towards UDP-glucose.

However, as shown in Table 1, after separation from the insoluble fraction by centrifugation, the supernatant fraction (considered as the middle layer) retained activity towards UDP-glucose and approx. 21% of activity to the untreated starch was re-

Table 1. Incorporation of [14 C]glucose from labelled sugar nucleotides into the soluble and insoluble fractions
Soluble and insoluble fractions were prepared as described in the text.

[14 C]Sugar nucleotide	Expt. no.	Incorporation of [14 C]glucose (c.p.m.) into:	
		Soluble fraction	Insoluble fraction
ADP-glucose	1	Trace	2537
	2	Trace	2225
	3	Trace	2086
UDP-glucose	1	1604	932
	2	1951	1251
	3	2610	2274

covered; activity towards ADP-glucose was lost. On the other hand, the insoluble fraction, which seems to represent morphologically a surface layer of the starch granule, had activity towards both ADP- and UDP-glucose.

The results indicate that the starch synthetase bound to the granule and having specificity towards UDP-glucose was solubilized by the urea/pullulanase method.

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