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Note

Treatment of amylomaize starch granules with urea: comparison with normal maize starch*†

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Two of the main features of starch granules are their insolubility in water and their very low susceptibility to the action of various enzymes. These properties are generally considered to be due to the formation of a stable hydrogen-bonded structure within the granules. Treatment with boiling water, alkali, acid, methyl sulfoxide, and urea are usually required to denature starch granules[1]. The interior of the starch granules can be solubilised by treatment with a high concentration of urea[2–5], and it has been suggested[2–4] that the solubilised part and the residual outside-layer are equivalent to the amylose and amylopectin fractions, respectively, in the original granules.

Amylomaize starch has a high content of amylose[6], and its properties and structure[7–16] are unique. However, there has been no report on the effect of urea on this anomalous starch. We now describe the solubilisation of granules of normal and amylomaize starch by treatment with urea and two starch-hydrolysing enzymes, namely, pullulanase (EC 3.2.1.41) and beta-amylase (EC 3.2.1.2).

Solubilisation of granules of normal maize starch increased with increasing concentrations of urea up to 10M (Fig. 1). With amylomaize starch, the amount of the solubilised carbohydrates was much lower than would be expected for the high content (47.5%) of amylose, which was determined by amperometric iodine titration[17]. Fig. 2 shows the time course of solubilisation of starch granules on treatment with 10M urea. The solubilities of normal and amylomaize starches remained approximately constant after treatment for 1 h, and the values were 23.0 and 4.0%, respectively, after treatment with urea for 3 h. The value for normal maize starch agrees well with its content (24.3%) of amylose.

*Dedicated to Professor Tatsuro Itoh on the occasion of his retirement from our laboratory.
†Structural Features of Amylomaize Starch, Part II. For Part I, see ref. 15.
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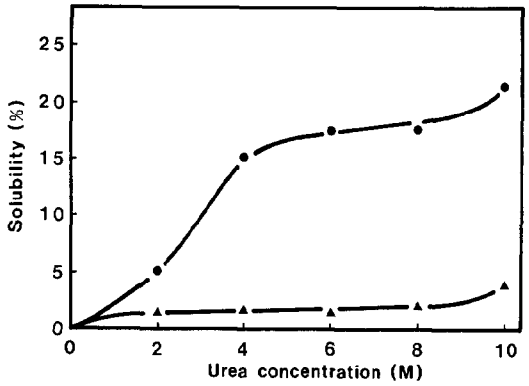

Fig. 1. Effect of urea concentration on solubilisation of starch granules. Normal (—●—) or amylomaize (—▲—) starch granules (10 mg) were treated with various concentrations of urea in a final volume of 1 mL for 2 h at 20°. The insoluble material was removed by centrifugation, and the amount of carbohydrate in the supernatant solution was determined by the phenol–sulfuric acid method.

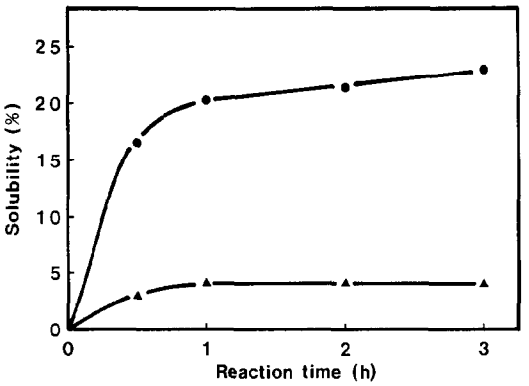

Fig. 2. Solubilisation of normal (—●—) or amylomaize (—▲—) starch granules (10 mg) in 10M urea (1 mL) at 20°. The solubilised carbohydrate was determined as described in Fig. 1.

The soluble and insoluble fractions of starch granules after treatment with 10M urea for 3 h were isolated and characterised (Table I). The urea-insoluble material of normal maize starch had very low affinity for iodine, whereas a high affinity and beta-amylolysis limit were found for the urea-soluble fraction, indicating that most of the amylose component in the starch granules of normal maize had been selectively solubilised by the treatment with concentrated urea. Also, the urea-soluble material of amylomaize starch had an affinity for iodine and a beta-amylolysis limit higher than those of the original starch and the urea-insoluble material. However, the iodine affinity of the urea-soluble fraction of amylomaize starch was smaller than that of the urea-soluble fraction of normal maize starch. This result implies that treatment of amylomaize starch granules with urea solubilises not only amylose but also other components in the original starch.

TABLE I

PROPERTIES OF UREA-SOLUBLE AND INSOLUBLE MATERIALS FROM NORMAL AND AMYLOMAIZE STARCHES

Polysaccharides	*λ_{max} of iodine complex (nm)*	*Iodine-binding capacity*[a]	*Amylose content (%)*	*Beta-amylolysis limits (%)*
Normal maize starch				
Original starch	600	4.6	24	63
Urea-soluble material	633	16.3	86	72
Urea-insoluble material	572	1.0	5	59
Amylomaize starch				
Original starch	602	9.0	47.5	66
Urea-soluble material	613	12.0	63	71
Urea-insoluble material	598	8.6	45	64

[a]Mg of iodine/100 mg of sample.

TABLE II

SOLUBILISATION OF MATERIAL INSOLUBLE IN 10M UREA ON TREATMENT WITH ENZYMES

Enzyme treatments[a]	*Solubilisation of insoluble material (%)*	
	Normal maize starch	*Amylomaize starch*
Pullulanase alone	60.0	1.7
Beta-amylase alone	40.8	1.5
Pullulanase plus beta-amylase	64.0	2.8

[a]Each enzyme treatment was performed at 30° for 3 h.

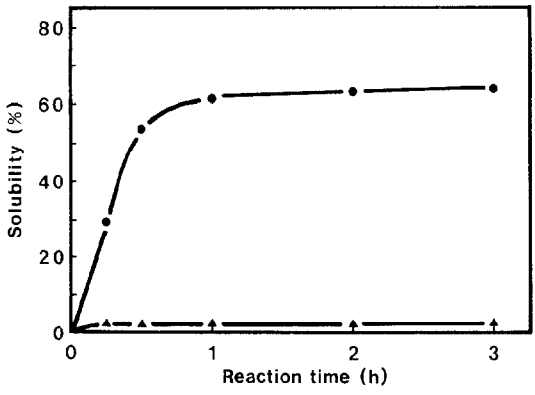

Fig. 3. Solubilisation of the material insoluble in 10M urea on treatment with pullulanase plus beta-amylase. Insoluble materials (10 mg) from normal (—●—) and amylomaize (—▲—) starch granules were each treated at 30° in a mixture (1 mL) containing 50mM acetate buffer (pH 5.5), 2 I.U. of pullulanase, and 50 I.U. of beta-amylase. Solubilised carbohydrate was determined as described in the Experimental.

Sasaki *et al.*[5] found that the insoluble fraction remaining after treatment of sweet-potato starch with concentrated urea was further solubilised on treatment with pullulanase. The fractions which remained insoluble after treatment with 10M urea for 3 h were treated with pullulanase plus beta-amylase (Fig. 3). Although ~30% of the insoluble material from normal maize starch was solubilised after only 15-min treatment with the enzymes, there was little solubilisation of the amylomaize starch even after 3 h. The extents of solubilisation were 64.0 and 2.8% for normal and amylomaize starches, respectively (Table II). These results indicate that ~72 and ~7%, respectively, of the original starch granules from normal and amylomaize were solubilised by the successive treatments with 10M urea and pullulanase plus beta-amylase. Also, extensive solubilisation of the urea-insoluble material from normal maize starch was observed after the respective treatments with these enzymes.

Thus, amylomaize starch granules have a high resistance to treatment with concentrated urea, as found for wrinkle-seeded pea starch[3], which also has a high content of amylose[18] and anomalous properties[13]. It may be concluded that solubilisation and/or swelling of starch granules on treatment with urea is not associated with the amylose content, but with the granular structure, although the amylose-like material is solubilised.

EXPERIMENTAL

Materials. — Amylomaize starch was prepared from 28-day-old endosperm tissues[19], according to the method of Schoch[20]. Normal maize starch and urea were obtained from Wako Pure Chemical Industries Ltd. (Japan). Crystalline *Aerobacter* pullulanase and sweet-potato beta-amylase were purchased from Hayashibara Biochemical Laboratories and Sigma, respectively. The beta-amylase was repurified[21] to remove contaminating α-D-glucosidase (EC 3.2.1.20). All other reagents were of the highest purities available.

Analytical procedures. — Absorption spectra of glucan–iodine complexes were recorded in the range 400–700 nm, using a Shimadzu UV-240 spectrophotometer[22,23]. Amperometric iodine-binding capacities of starches were measured[17,24] at room temperature. The limit of beta-amylolysis was determined in a digest containing polysaccharide (2 mg) dispersed in methyl sulfoxide, 50mM acetate buffer (pH 4.8), and 25 I.U. of beta-amylase in a total volume of 2 mL. After the hydrolysis was complete (12 h), the amounts of liberated maltose and total carbohydrate were measured by the copper reduction[25] and the phenol–sulfuric acid methods[26], respectively.

Preparation of urea-soluble and insoluble fractions of starch. — Defatted starch granules (2 g) were treated with 10M urea (200 mL) for 3 h at 20°, and the insoluble fraction was separated by centrifugation at 10,000*g* for 10 min and washed with water (100 mL) followed by centrifugation. The two supernatant solutions were combined, dialysed overnight against water (10 L), and then lyophilised

(yields: normal maize, 424 mg; amylomaize, 88 mg). The urea-insoluble fraction was further washed three times with water followed by centrifugation, dialysed against water (3 L), and lyophilised (yields: normal maize, 1.23 g; amylomaize, 1.48 g).

Enzyme treatments of urea-insoluble material. — The urea-insoluble material (10 mg) was treated in a mixture (1 mL) containing 50mM acetate buffer (pH 5.5) and pullulanase (2 I.U.) or beta-amylase (50 I.U.). After incubation at 30°, ice-cold water (4 mL) was added to each mixture, which was then centrifuged at 10,000*g* for 5 min. The amount of solubilised carbohydrates in the supernatant solution was measured by the phenol–sulfuric acid method[26], using maltose as the standard.

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