Structures of Amylopectin and Starch Granules: How Are They Synthesized ?

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Starch is synthesized by higher plants in nature and is produced as semi crystalline granules. Starch granules consist of highly branched amylopectin molecules with their external branch chains folded in double helical crystalline structure and primarily linear amylose molecules present in amorphous form. Consequently, waxy starch that consists of pure amylopectin displays a greater degree of crystallinity than the normal starch counterpart that consists of about 25% amylose. Because of the partially crystalline structure, starch granules are insoluble in water at temperatures below the gelatinization temperature of the starch.¹⁾ Under a polarized light microscope, starch granules display Maltese-cross patterns, indicating radial arrangements of amylose and amylopectin molecules in the granule. Amylose and amylopectin molecules in the granule are synthesized by apposition and oriented perpendicular to the surface of starch granules.

Relative locations of amylose and amylopectin molecules in the starch granule.

To investigate if amylose molecules are interspersed among amylopectin molecules or are present in bundles, chemical cross-linking reactions were applied to intact native starch granules. Results of the cross-linking reaction studies show that amylose molecules are cross-linked onto amylopectin molecules and amylopectin molecules are also cross-linked among themselves. No cross-links are found, however, between amylose molecules.^{2,3)} These results indicate that amylose molecules are interspersed among amylopectin instead of being present in bundles, as speculated for amylose based on its escaping from branching enzyme reactions. The fact that amylose and amylopectin molecules are interspersed in the granule and amylose is not subjected to the branching enzyme reaction, the mechanism can now be explained on the basis that the two molecules are synthesized side by side by two different starch synthases, granular bound starch synthase⁴⁾ and soluble starch synthase,⁵⁾ respectively.^{6–8)}

Is the internal structure of the starch granule homogeneous ?

To find out if structures and compositions of amylose and amylopectin are the same through out the granule (e.g., at the hilum and on the periphery), which are synthesized at different time order, Jane and Shen⁹⁾ peeled starch granules by layers to determine the structure at different radial locations. The authors used a surface gelatinization technique by treating native starch granules with saturated neutral salt solution, such as LiCl and CaCl₂, for different time periods to achieve different surface gelatinization percentages. Using this method, Jane and Shen⁹⁾ and Pan and Jane¹⁰⁾ reported that amylose is more concentrated on the periphery of the starch granule than at the core of the granule. This is consistent with the observation that starch granules of larger sizes (more mature) contain more amylose than do those of smaller sizes.⁹⁾ Branch chains of amylopectin molecules that are located at the core (around the hilum) of the granule are longer than that of the amylopectin located on the periphery. Scanning electron micrographs of the remaining starch granules after a substantial portion (up to 80%) of surface starch has been gelatinized and removed show that starch molecules around the hilum are in loosely packed amorphous structures.¹⁰⁾

Branch structures of amylopectin: How do they differ between the A- and the B-type crystalline starches ?

It is well established that the branch chain lengths differ between the A-type and the B-type crystalline starches;¹¹⁾ starch with longer branch chains displays the B-type X-ray diffraction pattern and that with shorter chains displays the A-pattern. Naegeli dextrans prepared by an extensive acid hydrolysis of various native starch granules also show different structures.¹²⁾ Naegeli dextrans of starches that display the B-type crystalline pattern consist of mostly linear chains (with a peak DP 13) and few branched chains (peak DP ~25). Whereas, Naegeli dextrans of the A crystalline-type starches consist of a large proportion of singly branched chains with a peak DP 25.¹²⁾ The authors interpret the results as that the amylopectin branch linkages of the B crystalline-type starches are more clustered and are located mostly in the amorphous

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region of the amylopectin molecule. Thus, the branch linkages are readily susceptible to acid hydrolysis and are mostly lost during the acid hydrolysis. In contrary, the amylopectin branch linkages of the A crystalline-type starches are scattered in both the amorphous and the crystalline region. Those branch linkages located in the crystalline region are protected from acid hydrolysis, and thus are preserved after the extensive acid treatments.

These proposed structures are in agreement with well-known facts that the A crystalline-type starches possess more short branch-chains and are more easily digestible by amylases than the B crystalline-type starches (Fig. 1). The proposed structures show that the B crystalline-type starches consist of double helices of branch chains that are more orderly aligned in the granule, and thus, they are more resistant to enzyme hydrolysis. The B crystalline-type starch granules (*e.g.*, potato) have a smooth surface without visible pinholes. This is attributed to the lack of weak points in the starch structure. The A crystalline-type starches are easily hydrolyzed by amylases, and the starch granules frequently display pinholes.¹³⁾ Animal-feeding studies using maize mutant starches of isogenic background also confirmed that starches consisting of shorter amylopectin branch chains, such as *sugary-2* maize starch, and starches of little or no amylose, such as waxy maize starch, are more easily digestible than the normal starch counter part.¹⁴⁾

Molecular weights and structures of amylopectin molecules of different origins.

Recent studies using high-performance size-exclusion chromatography have shown that amylopectin molecules of waxy starches display larger molecular weights than those of the normal starch counter parts.^{15,16)} Amylopectin molecules of the B crystalline-type starches, in general, have smaller molecular weights and lesser densities of dispersed molecules than that of the A crystalline-type crystalline-starches (Table 1 and Fig. 2).¹⁶⁾ The differences can be attributed to the following factors:^{15,16)}

1) The carbon flux of starch biosynthesis exclusively goes to amylopectin in waxy starch, but a portion (~25 %) of carbon flux goes to the biosynthesis of amylose in normal starch.

2) The A crystalline-type starch amylopectin consists of more short branch chains, which makes the molecules denser at the dispersed form. The B crystalline-type amylopectin carries less B1 chains, but more long chains that increase the gyration radius and result in lesser density of dispersed molecules.

3) Normal maize, barley, wheat, and other cereal amylopectin molecules carry extra-long branch chains,^{15,17)} but waxy amylopectin counterparts do not. The extra-long, amylose-like chains do not carry many other branch chains like a normal amylopectin branch chain and, thus, can reduce the molecular weight and the dispersed density.

Fig. 1. Relative enzyme digestibility of selected granular starches (uncooked) of different crystalline structures.

The letter above each bar indicates the type of crystallinity of the starch. Porcine pancreatic α -amylase (120 U/20 mg starch in 9 mL phosphate buffer, pH 6.9) was used for the study, and the enzyme reaction was carried out at 37°C for 24 h. The percentage enzyme hydrolysis was determined by measuring the total carbohydrate content in the supernatant using phenol-sulfuric acid method.

Table 1. Amylopectin molecular weights and gyration radii of selected starches.*¹

	$M_w (\times 10^8)^{*2}$	$R_z (\text{nm})^{*3}$	$\rho (\text{g/mol/nm}^3)^{*4}$
A-type starches			
Normal maize	4.9 (0.8) ^{*5}	312 (23)	16.1
Waxy maize	8.3 (0.2)	372 (11)	16.1
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Normal rice	26.8 (2.9)	581 (41)	13.7
Waxy rice	56.8 (9.3)	782 (36)	11.9
Sweet rice	13.9 (1.0)	486 (5)	12.1
Normal wheat	3.1 (0.3)	302 (3)	11.3
Waxy wheat	5.2 (0.4)	328 (6)	14.7
Barley	1.3 (0.1)	201 (8)	16.0
Waxy barley	6.8 (0.1)	341 (3)	17.1
Cattail millet	2.7 (0.2)	278 (6)	12.6
Mung bean	3.8 (0.2)	312 (3)	12.5
Chinese taro	12.6 (3.6)	560 (15)	7.2
Tapioca	0.7 (0.1)	191 (25)	10.0
B-type starches			
ae wx maize	3.2 (0.2)	306 (8)	11.2
Amylomaize V	2.4 (0.0)	357 (24)	5.3
Amylomaize VII	1.7 (0.0)	389 (57)	2.9
Potato	1.7 (0.2)	356 (36)	3.8
Waxy potato	2.0 (0.2)	344 (37)	4.9
Green leaf canna	3.4 (2.2)	436 (85)	4.1
C-type starches			
Lotus root	1.5 (0.4)	280 (57)	6.8
Water chestnut	7.1 (1.5)	230 (25)	58.4
Green hanana	1.9 (0.8)	286 (29)	8.1
Glycogen			
Cyanobacterial glycogen ^{*6}	0.2 (0.0)	55 (4)	99.2

*¹Data were averages of at least two replicates. *²Weight-average molecular weight. *³ R_z -Average radius of gyration. *⁴Density (ρ)= M_w/R_z^3 . *⁵Standard deviation. *⁶Glycogen was isolated from *Synechocystis* sp. PCC6803 in our laboratory.

Structural differences between the large and the small starch granules: How are they synthesized?

Barley and wheat starches are known to have two distinct groups of starch granules with different granule sizes: diameters of 15–32 μm for the barley large granule starch and 2–3 μm for the small granules (Figs. 3a, 3b and 3c). The large, A, granules display a disk shape, whereas the small, B, granules display spherical and irregular shapes. Both the large and small granule starches possess the A-type X-ray diffraction pattern. It has been reported by numerous researchers that the B granules display a higher gelatinization temperature (*ca.* 5°C at peak) than do the A granules (Table 2). Amylopectin molecular weights of the A and the B granules are 1.2×10^8 Da and 2.8×10^8 Da, respectively. Branch chain length distributions of the barley B starch granules showed more short chains (DP 6–12, 25.0%) and lesser B2 chains (DP 25–36, 14.7%) than did the A starch granules (22.6 and

Fig. 2. Relationships between the weight-average molecular weight (M_w) and z -average radius of gyration (R_z) of amylopectins.

Data are plotted on Log-Log scale. The linear regression line on the graph comprises data of A crystalline-type amylopectin from Ref. 16).

Fig. 3. Scanning electron micrographs of a) native, b) large granule and c) small granule barley starch.**Table 2.** Gelatinization properties of barley starches.*¹

Sample	Peak I ^{*2}				Peak II ^{*3}			
	T_o	T_p	T_c	$\Delta H(\text{J/g})$	T_o	T_p	T_c	$\Delta H(\text{J/g})$
Barley	57.9 $\pm$ 0.3	62.6 $\pm$ 0.1	67.9 $\pm$ 0.2	12.6 $\pm$ 0.3	94.1 $\pm$ 0.2	99.5 $\pm$ 0.5	103.3 $\pm$ 0.3	0.9 $\pm$ 0.1
Barley A type	57.0 $\pm$ 0.1	61.5 $\pm$ 0.4	66.4 $\pm$ 0.4	12.2 $\pm$ 0.7	94.1 $\pm$ 0.4	99.5 $\pm$ 0.4	103.4 $\pm$ 0.7	0.9 $\pm$ 0.2
Barley B type	58.2 $\pm$ 0.2	66.3 $\pm$ 0.4	73.6 $\pm$ 0.6	12.7 $\pm$ 1.2	94.4 $\pm$ 1.2	101.9 $\pm$ 1.2	109.2 $\pm$ 1.7	2.1 $\pm$ 0.2

*¹ T_o , T_p and T_c =onset, peak and conclusion temperatures (°C) of endotherm. ΔH =enthalpy change of gelatinization and melting of amylose-lipid complex. Values are mean $\pm$ SD. *²Gelatinization. *³Melting of amylose-lipid complex.

Fig. 4. High-performance anion-exchange chromatograms of debranched amylopectin of a) large (A) granule barley starch, b) small (B) granule barley starch, and c) differential histogram between B and A granules.

The debranched chains were separated by Dionex PA 100 column, treated with on-line amyloglucosidase, and detected by pulsed amperometric detector (HPAEC-ENZ-PAD).

17.1%, respectively) (Fig. 4 and Table 3). The fact that the large (A) granules consist of more long branch chain and lesser short chains than the small (B) granules but display a lower gelatinization temperature contradicts the current understanding of the relationship between branch chain length and gelatinization temperature. For most starches investigated, a starch that possesses more short branch-chains and less long branch-chains displays a lower gelatinization temperature.¹⁸⁻²²⁾ The histograms of branch chain length distributions of the two starches also showed that the A granule starch had a more distinct shoulder at DP 17–22. A differential histogram between the B granule starch and the A granule starch (Fig. 4c) showed that the B granule starch consisted of more branch chains of DP 6–12 and DP 16–21 but lesser chains of DP 22–38 than the A granule starch. Results from our previous studies indicate that a starch possessing lesser branch chains of DP 17–22 displays a lower gelatinization temperature.²¹⁾ This is attributed to the fact that the chain length of DP 17–22 is equivalent to the full length of the crystalline region of the amylopectin molecules. A starch that consists of fewer chains of the full length results in

Fig. 5. Proposed granular and molecular structures of small and large granule barley starch.

The granular structures are constructed on the basis of polarized optical map of each starch granule proposed by the late Professor Dexter French (Ref. 23).

Table 3. Branch chain-length distributions of amylopectins.

Sample	Peak DP		Distribution (%)				
	I	II	DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
Barley	12	46	4.9 ± 0.1	23.7 ± 0.7	50.1 ± 1.2	16.4 ± 0.2	9.8 ± 2.02
Barley A-type	12	46	4.5 ± 0.0	22.6 ± 0.1	50.9 ± 0.2	17.1 ± 0.1	9.3 ± 0.4
Barley B-type	12	44	5.7 ± 0.0	25.0 ± 0.1	50.9 ± 0.2	14.7 ± 0.2	9.5 ± 0.2

Fig. 6. Scanning electron micrographs of starch granules at different regions of a corn kernel.

Micrographs A-D show the starch granules located in each of the regions marked on the kernel.

defective crystals and lowers the gelatinization temperature.

Based on polarized optical map studies, arrangements of starch molecules in the disk-shaped granules were proposed.²³⁾ In a disk shaped granule, the majority of amylopectin molecules are oriented perpendicular to the flat surface of the granule. Thus, those molecules are parallel to one another. In a spherical granule, however, amylopectin molecules are oriented radially, which may give the molecules space to carry more short branch chains than those in the disk-shaped granule (Fig. 5).

Peng *et al.*²⁴⁾ reported that fractionated large A granules of wheat starch consist of proteins that have molecular weights of 140 and 145 Da. These proteins are less or not found in the small B granule fraction. The authors designate the proteins as a starch branching enzyme 1c (SBE1c). The authors propose that the SBE1c is missing in the B granule and that prohibits the further development of the small, B granules to large, A granules. It will be intriguing to investigate if the SBE1c is responsible for synthesizing the larger proportion of DP 22-38 chains in large granules. It is also interesting to investigate if the larger proportion of DP 22-38 chains and a lesser number of short A and B1 chains make the amylopectin molecules fits better to the parallel arrangement of amylopectin molecules for the disk-shaped A granules.

What is the nature of pinholes on the surface of starch granules ?

Pinholes are commonly found on the granule surface of A-typed crystalline starches, such as maize, sorghum, but not on the B-typed crystalline starches, such as the potato. It has been postulated that pinholes are the results of enzyme degradation of starch. A recent study using scanning electron microscopy has shown that maize kernels, at a dormant state, display significant enzyme attack on starch, particularly at the region of the germ. SEM micrographs of starch granules at different region of a kernel are shown in Fig. 6. These results suggest that starch is hydrolyzed by enzymes to produce energy for seed germination even at the dormant state. The enzyme reaction is the most active around the region of the germ. Starch granules with pinholes may be isolated from the region around the germ of the seed.

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