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Structure of Starch Granules

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Abstract: Starch granules are synthesized in a semicrystalline structure and have characteristic morphology and properties depending on the botanical origin and the organ of the plant. Surface gelatinization studies have shown that the internal structures of starch granules are not homogeneous and vary with radial locations, from the hilum to the periphery. The internal structure of maize starch granule coincides with the biosynthesis and development of starch granules and the expression of starch synthase activities during the maturation of maize kernels. The enzyme digestibility of starch granules relates to the internal structure and the internal surface area of the starch granule as revealed by confocal laser-scattering micrographs. Short branchchains of amylopectin (DP 6−12) destabilize the granule structures, resulting in lobed or compound starch granules and small granules. Ratios of long branch-chains to short branch-chains of amylopectin molecules affect the shape of the molecule, *e.g.*, cylindrical *versus* conical shapes. The shape of the amylopectin molecule may affect the packing of the molecules in the granule and, in turn, determines the morphology and the size of the starch granule.

Key words: starch, granule, structure, biosynthesis, enzyme digestibility

Starch is synthesized in semicrystalline granular structures, which is in contrast to glycogen that has an amorphous structure. The different structures of these two glucans could be related to the patterns of how these two storage glucans are utilized. Glycogen is present in muscle and liver, and it needs to be readily available to provide energy for muscle functions. Therefore, glycogen molecules are amorphous and are readily hydrolyzed to produce energy in animals.[1] Starch, however, is mainly synthesized in storage organs, such as seeds, tubers, and roots. To store glucan molecules in an effective and stable condition in those storage organs, starch is packed in the semicrystalline granular form to reserve energy for use when the seeds are ready to germinate and grow.[2]

Starch granules of different botanical origins display different morphology,[3] crystalline structures,[4,5] and susceptibility to enzymatic[6] and chemical reactions.[7] Experimental results have shown that these granular structures and properties are related to the structures of amylose and amylopectin synthesized in different plants,[4,5,8] different organs,[9,10,11] and at different stages of development.[9,12] In this lecture, the internal structures of starch granules and the concepts for how the molecules in the starch granule are synthesized will be reviewed. Also, data on how the structures of starch molecules affect the geometrical packing arrangements and, in turn, determine the shapes of starch granules will be discussed.

Structures and biosynthesis of starch molecules in the granule.

To gain a better understanding of the internal structures of starch granules, starch molecules on the surface of starch granules can be gelatinized using a saturated lithium chloride solution[13] or a saturated calcium chloride solution[14] and then separated from the inner part of the starch granule. Results obtained from the studies of surface gelatinization of potato[14] and normal maize[13] starches show that amylose is more concentrated on the periphery than in the core of the granule and the branch chains of amylopectin are shorter in the surface starch than that of the amylopectin in the remaining starch granule (Fig. 1). These results reflect that starch granules do not have homogeneous internal structures and suggest that starch biosynthesis is not consistent throughout the development of starch granules. The structures of amylose and amylopectin vary in the radial locations, from the hilum to the periphery.[13,14]

Recent studies[9] on structures of starch molecules during maize kernel development have shown that small starch granule nuclei (diameter 1−4 μm) first appear in the endosperm on 5 days after pollination (DAP), and the number of starch granule nuclei increases but the size remains similar up to 12 DAP.[9] On the 14 DAP, the sizes of starch granules appear larger (diameter ∼7 μm) and the sizes of the granules continue to increase up to 23 μm on 30 DAP.[9] These results are in agreement with the starch granule development in rice endosperm[12] and reported increases in the size of starch granules with kernel maturation of rice,[15] barley,[16] among other plants. These results indicate that the development of individual starch granule is parallel to the development of the kernels. During the maize kernel development, the starch content of the kernel increases from 1% on 8 DAP to 88.9% on 30 DAP, and the amylose content of the starch increases from 9.2% for the kernels harvested on 12 DAP to 24.2% on 30 DAP and remains similar for the mature and field-dried kernels harvested on 45 DAP (Table 1).[9] A substantial increase in

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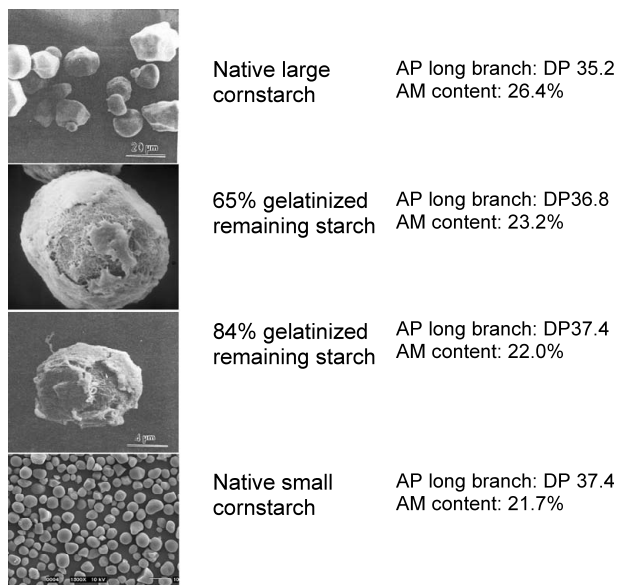
	Native large cornstarch	AP long branch: DP 35.2 AM content: 26.4%
	65% gelatinized remaining starch	AP long branch: DP36.8 AM content: 23.2%
	84% gelatinized remaining starch	AP long branch: DP37.4 AM content: 22.0%
	Native small cornstarch	AP long branch: DP 37.4 AM content: 21.7%

Fig. 1. Branch chain-lengths of the long chains of amylopectin and the amylose contents of native large-granule cornstarch (diameter >5 μm), 65 and 84% surface-gelatinized and removed remaining granules from the large granule cornstarch and native small-granule cornstarch (diameter < 5 μm).

The surface gelatinization is conducted using a saturated LiCl solution reported in Ref. 13).
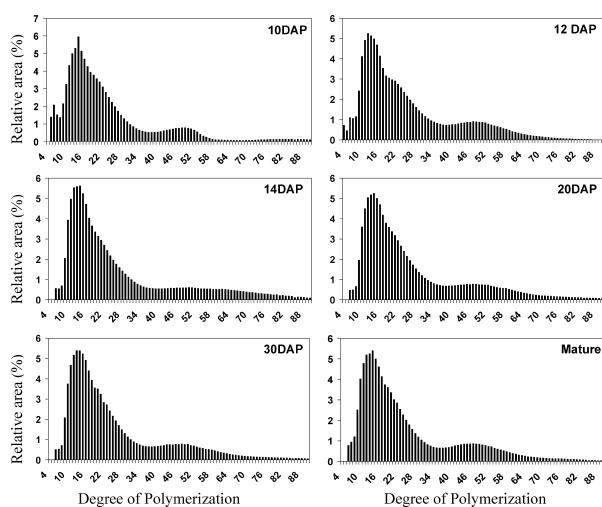

Fig. 2. Branch chain-length distributions of maize amylopectin isolated from starch at different maize kernel developmental stages, 10 days after pollination (DAP) to mature (45 DAP and dried).

The branch chain length of amylopectin is determined using isoamylase-debranched amylopectin and analyzed using high-performance anion-exchange chromatography equipped with an on-line glucoamylase reactor and a pulse-amprometric detector. A PA-100 anion-exchange column is used for the separation. Printed with permission from Ref. 9).

the starch content of maize kernels between 12 DAP and 14 DAP coincides with the increase in starch synthetic enzymes, including ADP-glucose pyrophosphorylase, UDP-glucose pyrophosphorylase, hexokinase, granule-bound starch synthases, and soluble starch synthases.[17] The increase in amylose content during the maturation of the kernel and starch granules coincides with the increase in the activity of granule-bound starch synthase (GBSSI).[10]

Table 1. Starch and amylose contents of the endosperm at different developmental stages of maize kernels.

Days after pollination	Starch content (% dry weight)	Amylose content (%)
0	ND[a]	ND
8	1.0±0.1	ND
10	1.5±0.8	ND
12	2.0±0.5	9.2±0.8
14	10.7±1.7	11.1±0.6
20	68.3±4.9	21.4±0.9
30	88.9±5.1	24.2±0.8

[a] Not determined.

Because starch granules are known to grow by apposition,[18,19] increasing the concentration of amylose would occur at the periphery of the starch granule. This result is in agreement with that obtained using surface-gelatinization method.[13,14]

Branch chain-length distributions of amylopectin molecules isolated from starch granules harvested at different maize kernel developmental stages show that the average branch chain lengths increase from DP 23.6 (10 DAP) to DP 26.7 (14 DAP) and then gradually decrease until mature (DP 24.9) (Table 2).[9] Short branch chains (DP≤12) of amylopectin also decrease from 10 DAP to 20 DAP and then increase until mature (Table 2 and Fig. 2).[9] Amylopectin molecules isolated from maize starch of 10 DAP show a large proportion of very short chains (DP 5−8), which diminish or reduce during the maturation of the maize kernels. The unusually large proportion of DP 5−8 may reflect the lack of SSI activity.[20] The amylopectin of 10 DAP also lacks long branch-chains larger than DP∼55 (Fig. 2), indicating the deficiency of soluble starch synthase activities. As the maize kernels develop to 12 DAP, the short branch chains of DP 4−8 decrease, whereas the long branch-chains (DP>55) increase and eventually reach the maximum length on 14 DAP (Fig. 2). There is an obvious shoulder shown at DP 17 in the branch chain-length distribution of amylopectin harvested on 12 DAP. This may suggest a deficiency in the activities of SSII and/or SSIII.[6,20]

When the maize kernels further develop and become more mature, the amylopectin branch chains of around DP 48 increase, but the branch-chains of DP>50 decrease as shown for the branch chain-length distribution of amylopectin of 20 DAP until mature (Fig. 2). The branch chain-length distribution of amylopectin harvested on 20 DAP and later dates show a typical bimodal branch chain-length distribution with the peak chain lengths at DP 14 and DP 48 for the short and long branch-chains, respectively. The increase in branch chains≤DP 24 after 12 DAP (Table 2) can result from the decrease in the ratio of BEI/BEIIb after 12 DAP.[21] The maize branching enzyme IIb (BEIIb) is known for transferring short branch-chains, whereas BEI is for transferring long branch-chains.[22,23] And the increase in branch chains of around DP 48 (Fig. 2) can result from the increase in SSIII activities and double helix formation.

The onset gelatinization temperature of the maize starch isolated from kernels of different development stages increases from 61.3°C (8 DAP) to the maximum of 69.0°C

Table 2. Branch chain-length distributions of endosperm amylopectins.

Samples	Percent distribution				Average CL
Days after pollination	DP≤12	DP 13−24	DP 25−36	DP≥37	
10	21.1±1.6[a]	48.3±0.2	14.2±0.0	16.3±1.8	23.6±0.9
12	21.1±0.1	42.2±0.1	15.8±0.4	20.9±0.2	24.8±0.1
14	18.3±0.1	45.6±0.2	13.7±0.5	22.5±0.7	26.9±0.2
20	16.7±0.0	46.2±0.1	15.1±0.3	22.1±0.3	26.3±0.1
30	17.4±0.5	47.5±0.9	14.5±0.3	20.6±1.6	25.4±0.6
45 (mature and dried)	19.4±0.0	46.3±0.8	13.4±0.3	20.8±1.0	24.9±0.5

[a]Values given are means±SD obtained from two replicates.

Table 3. Thermal properties of native endosperm starches.

Samples[a]	Native starch			
Days after pollination	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
8[b]	61.3	66.5	73.2	13.0
10[b]	61.5	66.9	73.3	13.3
12	63.0±0.2[c]	67.8±0.3	74.8±0.5	14.5±0.2
14	69.0±0.2	72.9±0.2	77.8±0.2	15.6±0.5
20	67.5±0.0	71.9±0.0	77.7±0.1	15.6±0.3
30	67.4±0.1	71.0±0.2	75.3±0.1	14.2±0.3
45 (mature and dried)	62.8±0.1	68.0±0.1	72.8±0.1	13.8±0.1

[a]Samples (∼3.0 mg, dsb) and deionized water (∼9.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively. [b]Value was measured once. [c]Values were calculated form three replicates; ±SD.

Fig. 3. Enzyme hydrolysis rates of maize mutant starches, normal, waxy, and sugary-2 maize starch, by porcine pancreatic alpha amylase. Printed with permission from Ref. 6).

(14 DAP), and then decreases to 67.4°C (30 DAP) and 62.8°C (45 DAP) (Table 3).[9] The mature and field- and air-dried starch granules (45 DAP), consisting of 19.4% short branch chains of DP≤12, display a substantially lower onset gelatinization temperature (62.8°C). This substantial decrease in the gelatinization temperature is interesting. Because the maize starch isolated from the mature and dried kernel of 45 DAP consists of a large number of pinholes on the surface of starch granules, further studies are needed to reveal if enzyme degradation of the starch during maturation may contribute to the reduction in the gelatinization temperature in addition to the presence of a large proportion of the short branch chains of DP≤12.[9]

Effects of the internal structure of starch granule on its enzyme digestibility.

Starch of different botanical origins is known to have different enzyme digestibility.[24−26] A feeding study with baby chickens using ground kernels of maize mutants, normal, waxy, and sugary-2 with isogenic background, show that the growth rate of the baby chickens fed with these three maize mutants decreases in the order of su-2 maize>waxy maize>normal maize.[6] The *in vitro* enzyme hydrolysis of the starch granules using porcine pancreatic α-amylase displays the same order (Fig. 3).[9] The differences in the enzyme digestibility between the three maize starch mutants can be attributed to their different molecular structures of amylopectin (Table 4) and amylose contents. Among the three maize starch mutants, the su-2 starch consists of amylopectin with a large proportion of short branch chains of DP 6−12 and a large content of apparent amylose (33.5%);[6] the waxy starch and the normal
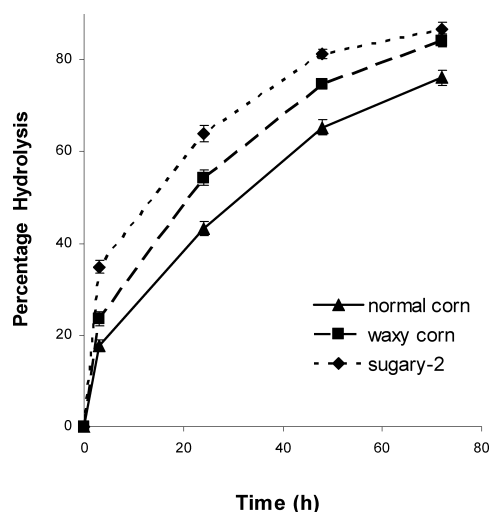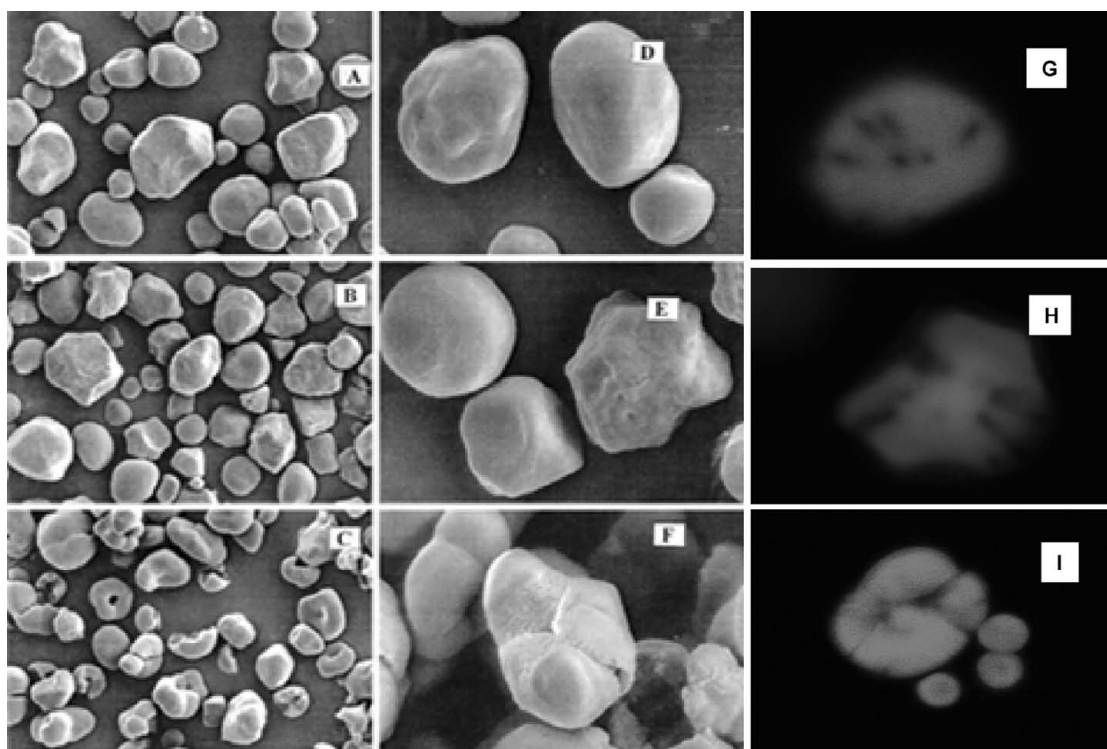
starch consist of amylopectin of similar structure, but the waxy maize starch has no amylose, whereas the normal maize starch has 21.6% amylose.[6] Because of the large content of short branch chains of amylopectin and a relatively large concentration of amylose, the starch granules of the su-2 maize starch display a lobe and compound granule morphology, as shown by scanning electron micrographs (SEM) (Fig. 4).[6] The su-2 maize starch also displays weak crystallinity and a very low gelatinization temperature, 47.8°C *versus* 64.4°C for normal maize starch and 64.2°C for waxy maize starch.[6]

The maize su-2 mutant is known for missing soluble starch synthase II (SSII) activity, which results in a very large proportion of short branch chains of DP 6−12 (22.2%) and fewer chains of DP 25−36. The large proportion of short branch chains (A and B1 chains) and lacking of B2 chains to connect between two clusters result in amylopectin molecules with brush-like cluster structure, which do not form extended and stable double helices and, thus, display low crystallinity, low gelatinization temperature, and even lose the integrity of starch granules. Potato starch of SSII and SSIII activity missing also show lobe and compound starch granules,[27,28] but when both SSIII and GBSS activities are missing, the potato starch granules develop into a regular shape.[28] Amylopectin of naturally present compound starches, such as rice and oats starches, have large proportions of short branch chains of DP 6−12.[8] The relationship between the granular structure formation and the short branch chains of DP 6−12 is in-

Table 4. Branch chain length distribution of amylopectin.[a]

Starch	Peak 1	Peak 2	% Distribution (DP)				
			6−9	6−12	13−24	25−36	≥37
Normal corn	13	45	2.9±0.3	15.0±0.4	48.0±0.7	15.5±0.5	21.4±0.2
Waxy corn	14	49	3.9±0.2	14.6±0.1	47.9±0.5	18.5±0.5	19.4±0.5
Sugary-2	13	49	6.5±0.2	22.2±0.3	44.4±0.2	12.8±0.1	20.4±0.3

[a]Results were obtained by using a high performance anion exchange chromatograph equipped with a post column amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD), mean±SD.

Fig. 4. Scanning Electron Micrographs (SEM) and confocal laser-scattering micrographs (CLSM) of maize mutant starches.

A and D are SEM of normal maize starch at different magnification; B and E, SEM of waxy maize starch; and C and F, SEM of sugary-2 maize starch. G, H, and I are CLSM of normal, waxy, and sugary-2 maize starch, respectively. Reprint with permissions from Refs. 6) and 29).

triguing and needs further studies.

Confocal laser-scattering micrographs (CLSM) of a su-2 maize starch granule stained with Rhodamin B fluorescent dye and extra dye removed show separate aggregates of starch sub-granules in the granule.[29,30] The small sub-granules hold the dye tightly (Fig. 4). Whereas that of the normal maize starch granules display channel-like voids and that of waxy maize starch granules show a loosely packed structure at the periphery. The difference in the CLSM images between the normal maize and waxy maize starch can be attributed to the presence of amylose in the normal maize starch, but not in the waxy maize starch. Amylose is known to be more concentrated at the periphery of the starch granule. The amylose in the normal maize starch intertwines with amylopectin and holds the molecules together, particularly at the periphery of the granule where amylose is more concentrated. The intertwining between amylose and amylopectin maintains the integrity of the starch granules.[31] Waxy maize starch does not have amylose to intertwine with amylopectin and, thus, displays a loosely packed structure at the periphery. Consequently, waxy maize starch granules tend to disperse more easily[31] and show fission when it is suspended in DMSO.

All three maize mutant starch granules show internal surface,[29,30] which results in greater susceptibility to enzyme hydrolysis[6] and chemical penetration[7] when compared with the starches of the B-type polymorphism and some C-type polymorphism.[30] Among these three starches, su-2 starch consists of the largest internal surface, which displays the greatest enzyme digestibility, followed by waxy maize starch and normal maize starch, as shown in Fig. 3.[6] The internal voids shown in CLSM images coincide with the internal structures of starch granules revealed after surface starch has been removed by surface gelatinization. The remaining starch granules of normal maize starch show a porous and loosely packed or split structure, whereas that of the potato starch show a solid structure.[30]

Relationships between starch molecular structures and granular shapes.

Starch of different botanical origins display characteristic and diverse granule shapes and sizes.[3] For examples, wheat starch consists of bimodal granules: large and disk shaped A-granules and small and spherical B-granules
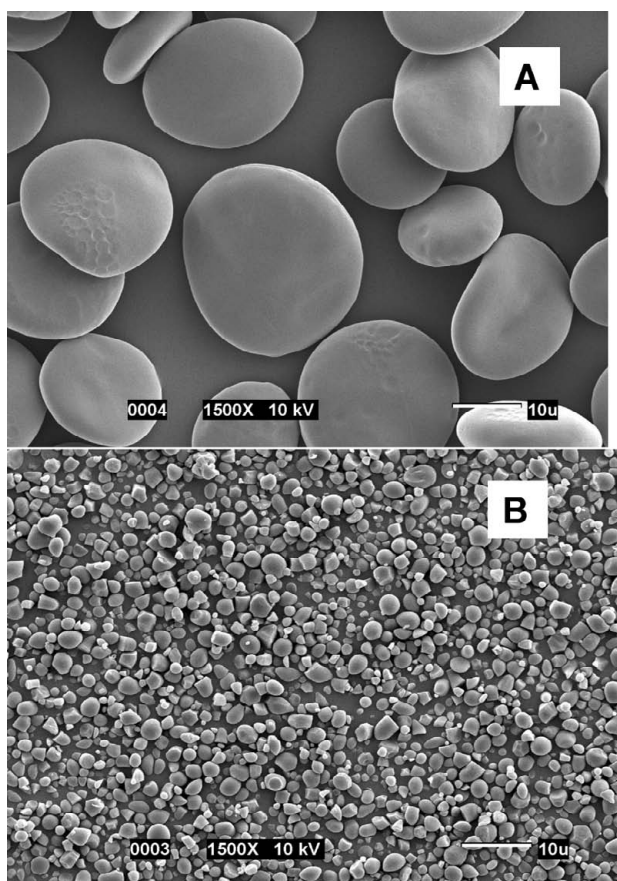
Fig. 5. Scanning Electron Micrographs (SEM) of fractionated wheat large-granule starch (A) and wheat small-granule starch (B).

(Fig. 5).[32] It is not known how starch granules are synthesized into specific and characteristic shapes for different plants and in different organs. Some recent studies have shown that the branch structures of amylopectin molecules of the wheat A-granule differ from that of the B-granule.[32] The amylopectin of the large and disk-shaped A-granules consists of more B2 chains that extend through two clusters, whereas that of the spherical B-granules consists of more short chains, *i.e.*, A chains and B1 chains that extend only through one cluster (Fig. 6). When there are more B2 chains present in the molecules, the amylopectin molecules have cylindrical shapes and are likely to arrange in a parallel pattern and developed into a disk-shaped granule as revealed by an optical density map.[33] In contrast, when an amylopectin molecule consists of more short chains, A and B1 chains, and fewer B2 chains, the molecule has a conical shape and is better arranged into a spherical granule, the B-granule. Further studies are needed to fully understand the effects of molecular structures on the granular structures.

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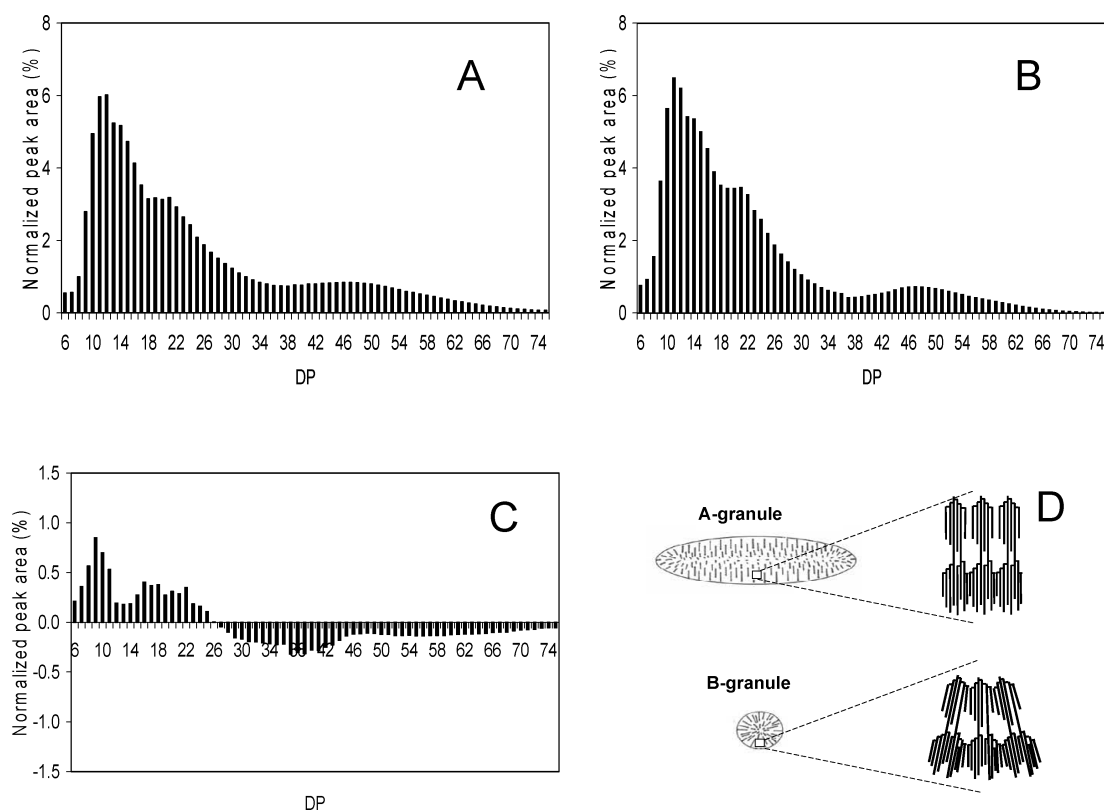

Fig. 6. Branch chain-length distribution of amylopectin isolated from wheat large-granule starch (A), and wheat small-granule starch (B), the difference in branch chain-length between the large and the small granule starch (B-A) (C), and proposed models of the amylopectin molecules of the A and the B granules.

The branch chain-length analysis is conducted using the same method described in Fig. 2. Printed with permission from Ref. 32).

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