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Mini Review

Towards a Better Understanding of the Metabolic System for Amylopectin Biosynthesis in Plants: Rice Endosperm as a Model Tissue

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Starch is made up of amylose (linear α-1,4-polyglucans) and amylopectin (α-1,6-branched polyglucans). Amylopectin has a distinct fine structure called multiple cluster structure and is synthesized by multiple subunits or isoforms of four classes of enzymes: ADPglucose pyrophosphorylase, soluble starch synthase (SS), starch branching enzyme (BE), and starch debranching enzyme (DBE). In the present paper, based on analyses of mutants and transgenic lines of rice in which each enzyme activity is affected, the contribution of the individual isoform to the fine structure of amylopectin in rice endosperm is evaluated, and a new model referred to as the "two-step branching and improper branch clearing model" is proposed to explain how amylopectin is synthesized. The model emphasizes that two sets of reactions, α-1,6-branch formation and the subsequent α-1,4-chain elongation, are catalyzed by distinct BE and SS isoforms, respectively, are fundamental to the construction of the cluster structure. The model also assesses the role of DBE, namely isoamylase or in addition pullulanase, to remove unnecessary α-1,6-glucosidic linkages that are occasionally formed at improper positions apart from two densely branched regions of the cluster.

Keywords: Amylopectin — Endosperm — Rice — Starch.

Abbreviations: BE, starch branching enzyme; DBE, starch debranching enzyme; DP, degree of polymerization; GBE, glycogen branching enzyme; GS, glycogen synthase; SS, soluble starch synthase.

Introduction

Amylopectin accounts for about 65–85% of storage starch and has a defined structure composed of tandem linked clusters (approximately 9–10 nm each in length) where linear α-1,4-glucan chains are highly and regularly branched via α-1,6-glucosidic linkages, whereas glycogen of bacteria and animals has a randomly branched structure (Fig. 1) (see Thompson 2000). Amylopectin, as compared with glycogen, appears to be a product refined by evolution so that it fits the survival strategy of plants. For example, amylopectin forms starch granules in the cell and this enables cells to store larger amounts of glucose molecules with lesser effects on internal osmotic potential as compared with glycogen. In the cluster structure of amylopectin, A chains carry no chains and are linked to the other chains at their reducing end glucose units, while B chains carry one or more chains. The only chain that contains a reducing terminal in an amylopectin molecule is called the C chain (Peat et al. 1952). Hizukuri (1986) proposed that B chains that are present within a single cluster are designated as B_1 chains, whereas long B chains interconnecting the clusters are referred to as B_2 and B_3 chains, depending on the number of clusters interconnected.

The multiple cluster structure of amylopectin exhibits two regions that are repeated, the amorphous lamellae and the crystal lamellae. The formation of double helices by adjacent side chains with degree of polymerization (DP)≥10 is promoted by their parallel alignment. The double helices then become organized in regular arrays, and these arrays form the crystalline lamellae observed within the granule (Fig. 1). The distinct structure of amylopectin contributes to the crystalline organization of the starch granule (Gallant et al. 1997). Variations in the fine structure of the cluster cause profound variations in starch functional properties between species (e.g. corn starch vs. potato starch), tissues (e.g. storage starch vs. assimilatory starch) and genetic backgrounds (e.g. Japonica rice starch vs. Indica rice starch).

Recent investigations have shown that amylopectin is synthesized via concerted reactions catalyzed by four classes of enzymes, i.e. ADPglucose pyrophosphorylase (AGPase), soluble starch synthase (SS), starch branching enzyme (BE), and starch debranching enzyme (DBE). AGPase has large and small subunits, and the other enzymes are composed of multiple isoforms encoded by different genes (Fig. 1). For example, our preliminary EST (expressed sequence tag) analysis reveals that rice endosperm has at least one small and two large subunits of AGPase; BEI, BEIIa and BEIIb isoforms; SSI, SSIIa, SSIIIa and SSIIIb isoforms; and two DBEs, isoamylase and pullulanase.

The mechanism of how the amylopectin cluster structure

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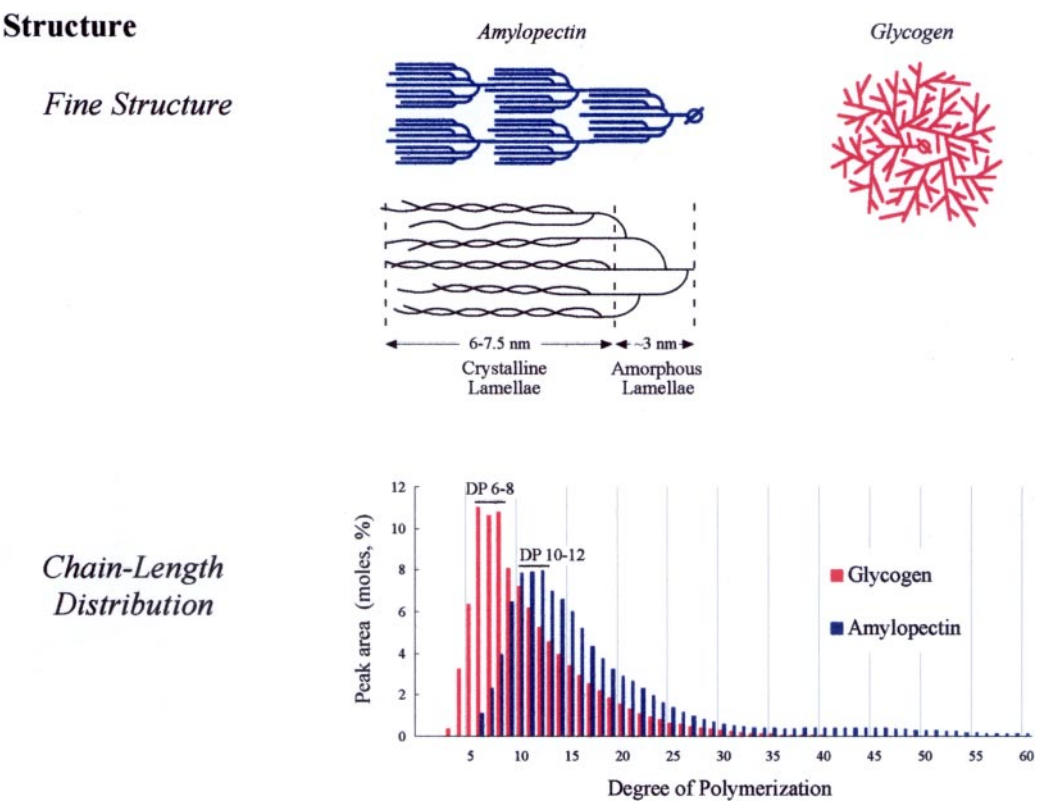
is synthesized is not yet well understood although several models have been proposed (Ball et al. 1996, Smith et al. 1997, Zeeman et al. 1998, Myers et al. 2000). Although all the amylopectin synthesizing isoforms so far examined have distinct structures and functions, these models do not fully explain the specific contribution of the individual isoform to the fine structure of amylopectin. In this paper, the role of each isoform in determining the amylopectin structure in rice endosperm will be discussed, and a new model for explaining the involvement of the enzyme in amylopectin biosynthesis will be proposed.

Contribution of each enzyme to the fine structure of amylopectin

Starch branching enzymes (BE)—BE is the only enzyme that can introduce α-1,6-glucosidic linkages into α-polyglucans in plants. Plants have two types of BE, namely BEI and BEII. Some cereals possess the distinct isoform BEIIb that is specifi-

	Plants	*Bacteria*	*Animals*
Polyglucan	**Amylopectin**	**Glycogen**	**Glycogen**
Biosynthesis			
Donor Formation	AGPase Small subunit Large subunit	AGPase	UGPase
Chain Elongation	SSI SSII SSIII	GS	GS
Branching	BEI BEII	GBE	GBE
Debranching	ISA PUL (?)		



cally expressed in the endosperm, in contrast to the other form, BEIIa, which is present in every tissue (Yamanouchi and Nakamura 1992).

It should be noted that plant BEI and BEII have distinct properties in preference for α-1,4-chain lengths. For example, maize endosperm enzymes can produce chains with a wide range of chain-lengths over DP33 as compared with glycogen branching enzyme (GBE), which effectively forms a narrower range of short chains of DP6–20 when these enzymes are incubated with amylose of DP405 (Takeda et al. 1993, Guan et al. 1997). It should also be pointed out that maize BEI preferentially produces longer chains whereas BEII generates shorter chains, and that the minimum chain length required for BEI is presumably DP16 while that for BEII is DP11–12, the same value for GBE (Takeda et al. 1993, Guan et al. 1997).

Nishi et al. (Nishi et al. 2001 and unpublished) have succeeded for the first time in isolating mutants of rice lacking in three individual BE isoforms in the endosperm. Biochemical analyses of BEIIb-deficient mutants (*amylose-extender*, *ae*) revealed that the reduction in BEIIb leads to a specific decrease in short chains of DP≤13, with the greatest decrease in chains of DP8–11 (Fig. 2A). The gene dosage experiment showed that in mutants where the levels of BEI and BEIIa are not altered, the extent of the change in chain lengths is related to the decrease in BEIIb activity (Nishi et al. 2001). The results strongly suggest that BEIIb plays a distinct role in the formation of A chains, and the role cannot be complemented by BEIIa and/or BEI.

Fig. 2A shows that when BEI is lacking, amylopectin contains fewer intermediate-size (16≤DP≤23) and long chains (DP≥37) and more short chains (DP≤12) although the extent of the changes is not as marked as when BEIIb is lacking. The results suggest that BEI plays an important, but not exclusive, role in the synthesis of B_1 chains and B_2-B_3 chains.

Although BEIIa activity accounts for about 15–20% of the total BE activity in rice endosperm, which is similar to the contribution of BEIIb (Yamanouchi and Nakamura 1992), the BEIIa-deficient mutant exhibits no significant change in the amylopectin chain profile (Fig. 2A). However, this does not necessarily mean that the enzyme is useless; but, it is more likely that BEIIa supports the function of BEIIb and/or BEI in the rice endosperm. In fact, our preliminary results showed that when BEIIa activity is inhibited, levels of short-chains with DP≤10 decrease significantly in the amylopectin of the rice leaf sheath where BEIIb is not expressed. This result is consistent with the report of Blauth et al. (2001) that in *Mutator* insertional maize mutants of BEIIa, short chains of amylopectin are much diminished in leaf starch while the chain-profile for amylopectin of kernel starch is indistinguishable between the mutant and wild-type, suggesting that BEIIa plays a distinct role in the synthesis of short chains of amylopectin in the assimilatory starch.

Soluble starch synthases (SS)—Starch synthase catalyzes the chain-elongation reaction of α-1,4-glucosidic linkage by transferring a glucose moiety from ADP-glucose to the non-reducing end of the linkage in plants. Plants have at least three types of SS, namely SSI, SSII, and SSIII. The relative activities of these isoforms greatly vary depending on the plant species and tissues (Smith et al. 1997).

Recently we found that the structure of amylopectin from cultivated rice varieties can be classified into either the L-type or the S-type (Umemoto et al. 1999, Nakamura et al. 2002). The L-type amylopectin produced in many Indica-type varieties can be distinguished from the S-type amylopectin found in most Japonica-type varieties in that the proportion of short chains of DP≤10 in the former is specifically lower than that in the latter (Fig. 2B, D). Gene mapping analysis strongly suggested that the *SSII* gene is responsible for the structural difference between both types of amylopectin, and that the capacity of SSIIa for elongation of A (plus B_1) chains is defective in the S-type amylopectin-producing rice varieties (Umemoto et al. 2002).

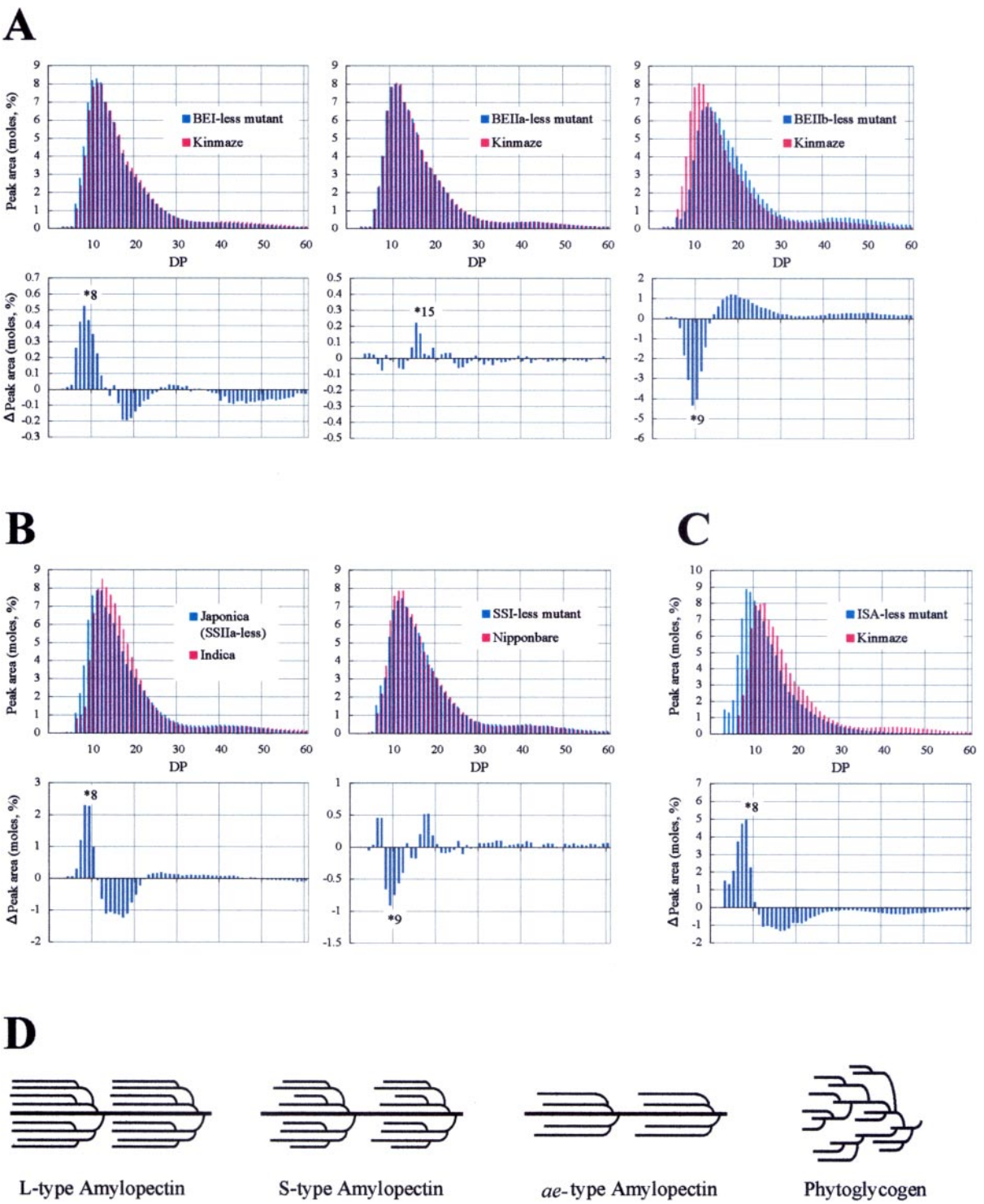
Analysis of a rice mutant with a retro-transposon inserted into a gene encoding SSI indicated that the mutant amylopectin is depleted in chains of DP8–12 and enriched in chains of DP6 and 7 (Fujita et al. unpublished; Fig. 2B), suggesting a distinct capacity of SSI for the synthesis of chains with DP8–12 from DP6–7.

No information is available on the effect of the reduction of SSIII activity on the structure of amylopectin in rice, while

Fig. 1 The structure of branched α-polyglucans occurring in organisms and their synthesizing enzymes. Upper panel: Enzymes involved in amylopectin synthesis in plants and glycogen synthesis in bacteria and animals. Note that the amylopectin synthesis system contains multiple forms of the enzymes and starch debranching enzymes, isoamylase and pullulanase, although involvement of pullulanase is not directly proven. AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase; SS, soluble starch synthase; GS, glycogen synthase; BE, starch branching enzyme; GBE, glycogen branching enzyme; ISA, isoamylase; PUL, pullulanase. Lines show α-1,4-glucan chains. Φ shows a reducing terminal glucose. Lower panel: Schematic representation of α-polyglucans. In amylopectin numerous structural units called clusters are tandem linked, and α-1,6-glucosidic linkages are localized in the amorphous lamellae and crystalline lamellae of the cluster whereas glycogen branches are randomly distributed throughout the spherical molecule and are more frequently present than amylopectin branches. In amylopectin cluster, longer chains of DP≥10 form double helices. Chain-length distribution of constituent α-1,4-glucans in amylopectin from the endosperm of Japonica rice is compared with that in glycogen from a cyanobacterium, *Synechococcus*. Note the polymodal distribution of chains in amylopectin, especially long cluster-linking chains of DP35–50 forming a small but distinct second peak besides a large peak containing A and B_1 chains. In glycogen, most short chains form a large single peak and no second peak is found, indicating the absence of the cluster structure. In addition, peak chain lengths in the large peak are apparently shorter in glycogen (DP6–8) than in amylopectin (DP10–12).

lesion of the gene coding for SSIII (*Dull1* gene) in maize (Gao et al. 1998) causes accumulation of approximately 15% of the starch as intermediate-size highly branched polyglucans (Inouchi et al. 1987, Wang et al. 1993).

Little is known about the specificities for glucan chain-lengths of plant SS isoforms. Using *Escherichia coli* transformed with maize *BEI*, *BEII* and various *SS* genes as well as *E. coli GS* gene, Guan and Keeling (1998) reported that SSI, SSIIa and SSIIb can synthesize a wide range of α-1,4-chains up to about DP40, while *E. coli* GS preferentially produces short chains of DP5–25. The results suggest that plant SS isoforms have a feature to construct various lengths of chains



ranging from short to long chains observed in amylopectin. However, it is also striking to note that maize SSI preferentially synthesizes short chains of DP6–15 whereas maize SSIIa preferentially produces longer chains of DP>24. The specific contributions of SSII and SSIII isoforms to the structure of amylopectin in potato tubers were extensively studied by Edwards et al. (1999) using antisense technology. Their data showed that depletion of long chains (DP25–35) in amylopectin is more pronounced under reduced SSIII activity rather than reduced SSII activity, suggesting that SSIII preferentially synthesizes long B_1 and B_2 chains as compared with SSII.

Although more precise experiments are needed to characterize the role of each SS isoform in amylopectin biosynthesis, current evidence supports the view that SSI, SSIIa, and SSIII specifically contribute to the synthesis of very short chains, A + B_1 chains, and long B_1 and B_2 chains of amylopectin, respectively.

Starch debranching enzymes (DBE)—DBEs that directly hydrolyze α-1,6-glucosic linkages of α-polyglucans are divided into two types, isoamylase and pullulanase. They differ in substrate specificity, i.e. isoamylase debranches glycogen, phytoglycogen and amylopectin, but scarcely attacks pullulan, whereas pullulanase can attack pullulan and amylopectin, but not glycogen and phytoglycogen.

It has been long accepted that the role of DBE is restricted to the debranching of amylopectin during complete hydrolysis of starch such as in germinating seed. However, several recent investigations revealed that lesion of the isoamylase gene causes a dramatic change of amylopectin into randomly and more highly branched polyglucans, often referred to as phytoglycogen in endosperm of maize (James et al. 1995) and rice (Nakamura et al. 1996), in *Arabidopsis* leaf (Zeeman et al. 1998) and in *Chlamydomonas* (Mouille et al. 1996). These observations can be reasonably explained by assuming that isoamylase plays an essential role in amylopectin biosynthesis and that, in the absence of the enzyme, the highly ordered structure of amylopectin is replaced by phytoglycogen or modified amylopectin (Fig. 2C, D). The idea is also supported by our preliminary result (Kubo et al. unpublished) that the wheat *Isoamylase* gene expressed in rice *sugary-1* mutants enables them to replace phytoglycogen with starch in the endosperm.

Nakamura et al. (1997) and Kubo et al. (1999) previously reported that some lines of rice *sugary-1* mutant, which are defective in the *Isoamylase* gene, accumulate only phytoglycogen in the whole endosperm, whereas the other mutant lines contain both PG-cells, in which starch is completely replaced by phytoglycogen, and AP-cells, which contain *sugary*-amylopectin (with more short chains than normal amylopectin) and amylose, in the inner and outer regions of the endosperm, respectively. They also found that the proportion of AP-cells to PG-cells is closely related to the level of pullulanase activity, but not to isoamylase activity. These results suggest that both DBEs are involved in the synthesis of amylopectin in rice.

In all *sugary-1* mutants of rice, the activity of isoamylase is at most or much lower than 1% of the wild type. Recently, Fujita et al. (unpublished) observed that when isoamylase activity is reduced to about 6% of that of the wild type by antisense strategy, PG-cells are absent in the antisense endosperm but amylopectin is converted into *sugary*-amylopectin-like insoluble polyglucans (84%) and phytoglycogen-like soluble polyglucans (about 16%). Since there is no significant inhibition of pullulanase in the transformant, one likely possibility is that PG-cells occur when both DBEs are defective, although *sugary*-amylopectin as well as phytoglycogen can be formed in the endosperm cell when isoamylase is solely inhibited. To explain these phenomena, two threshold values for isoamylase activity, T1 and T2, are hypothesized. When isoamylase activity in a single rice endosperm cell is depressed below the T1 value, slightly higher than 6% of the wild-type activity, normal amylopectin is significantly altered to be *sugary*-amylopectin as a major component and phytoglycogen as a minor component. It is likely that pullulanase is ineffective in converting *sugary*-amylopectin to normal amylopectin. By contrast, a dramatic change in amylopectin happens when isoamylase activity is reduced below the T2 value, approximately 1% of the wild-type level. Starches are totally replaced by phytoglycogen in cells (PG-cells) when pullulanase activity is also depressed. When pullulanase activity is high, cells (AP-cells) accumulate *sugary*-amylopectin, if not exclusively, indicating that pullulanase can supplement the function of isoamylase to some extent.

Model for the synthesis of amylopectin in rice endosperm

Jane et al. (1997) showed that the branch points are distributed both in the amorphous lamellae and crystalline lamellae of amylopectin at least in A-type starches (Fig. 1). Bertoft and Koch (2000) proposed that the crystalline lamellae of rice endosperm amylopectin contain chains with a maximum length of DP about 17, including A chains and the short B_1 chains.

Fig. 2 Comparison of the structure of amylopectin in the rice endosperm from various mutants and varieties. The upper panels show chain-length profiles and the lower panels, differences in chain-length profiles. The number of individual chains is expressed as a percentage of the total branch chains of DP≤60 on the molar basis. The rice varieties and mutants used in the figure are shown in the panels, and they all belong to the Japonica variety except for the Indica variety Co13. The data for mutants are shown with their parent cultivars. (A) BEI-, BEIIa- and BEIIb-deficient mutants (unpublished data from Satoh and Nakamura). (B) SSIIa-less Japonica rice (Nakamura et al. 2002) and SSI-deficient mutant (unpublished data from Fujita and Nakamura). (C) An isoamylase-deficient *sugary-1* mutant. (D) Schematic representation of the structure of amylopectin cluster and phytoglycogen. It is noted that the difference between L-type and S-type amylopectins of Indica and Japonica rice, respectively, occurs in chain-lengths within the cluster, but not in the branch positions and frequency (Nakamura et al. 2002), and that in *ae*-amylopectin, the number of chains branched in the crystalline lamellae is specifically reduced (Nishi et al. 2001), whereas phytoglycogen lacks the cluster structure (Kubo et al. 1999).

Based on all the results described above, I propose a model for amylopectin biosynthesis in rice endosperm, which could be called the "two-step branching and improper branch clearing model" (Fig. 3).

(1) Branching in the amorphous lamellae, followed by elongation of branched chains for synthesis of the core skeleton of the new cluster—When chains included in the preceding cluster are elongated to reach the length of the cluster-size, new chains are generated either on a B_2 chain or on the longest chain of the cluster by the action of BE (Fig. 3-II). It is assumed that transferred chains are derived from longer chains in the preceding cluster and/or from those in the neighboring cluster(s). It is likely that BEI plays the most important role in the branching reactions whereas BEIIa and BEIIb might share the function to some extent. Most branching points are localized in the restricted area that is destined for an amorphous lamella, although a few branches may be formed away from such a densely branched zone. It is noted that DBE plays a crucial role in removing the few branched chains that will eventually be improperly positioned in the newly synthesized cluster if they remain untreated (Fig. 3-II). The resulting and transferred chains are most effectively elongated presumably by SSIII or in addition by SSI and SSIIa (Fig. 3-III).

(2) Branching in the crystal lamellae, followed by elongation of chains for the completion of the new cluster—When these chains reached around DP12, the minimum chain length for branching by BEII, BEIIb acts on them to produce dense branches at the region slightly but distinctively apart from the previous branched region (Fig. 3-IV). A small number of branches, if positioned remotely from the second highly branched zone, will be efficiently hydrolyzed by DBE. SSIIa is considered to play a major role in elongating the newly formed short chains, although SSI might be partly involved in this process by elongating very short chains (Fig. 3-V). Thus, the synthesis of a new cluster is completed (Fig. 3-VI).

During the whole process, DBEs play critical roles in trimming the cluster shape. The timing of clearing the improper branches is very important because it is difficult to remove them after the synthesis of the cluster, and their presence will interfere with the regular array of chains in the cluster. Although both isoamylase and pullulanase have capacities for attacking most, if not all, of the branches of amylopectin during a prolonged time, they can act on sparsely located branches more quickly than on densely packed branches. This property is of great advantage in clearing the improper chains branched dispersedly during the branching reactions. The differences in the substrate specificity and minimum number of branched molecules for debranching between isoamylase and pullulanase would also be effective in promptly clearing various lengths of short chains.

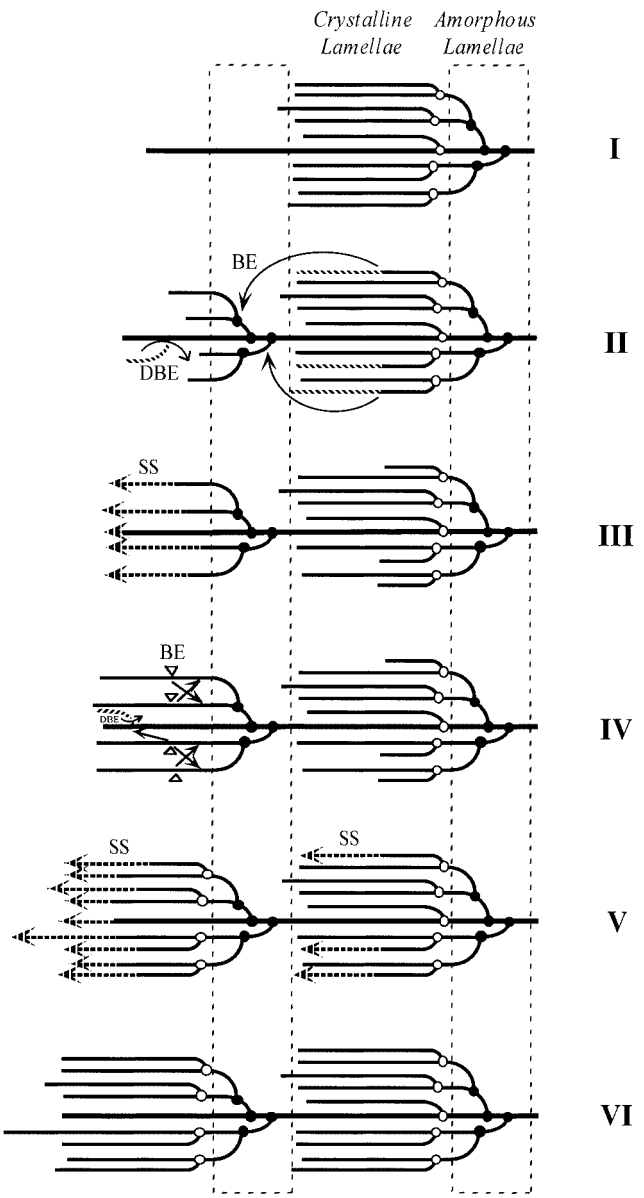


Fig. 3 A model for the synthesis of amylopectin cluster in rice endosperm. (I) When a unit cluster of amylopectin is completed, a B_2 chain or the longest chain in the cluster is ready to receive branches for the synthesis of the subsequent cluster. (II) Most of several branches are localized in a comparatively narrow range. The branched region will finally be in the amorphous lamella. It is noted that a few branches formed remotely from the region are cleaned out by DBE. (III) Chains are elongated by SS activities. SSIII or in addition, SSI possibly predominates in the reaction. (IV) When the lengths of chains reach DP of 12 or longer, BEIIb acts specifically on these chains to make many branches in the restricted region. DBE also plays an important role to remove a few chains branched in an improper position. The triangle (∇) and arrows show the sites for the cleavage and the subsequent branching of chains by BEIIb, respectively. (V) The resultant chains including newly formed chains and SSIIa predominantly elongates donor chains, although SSI probably plays a supporting role by elongating very short chains to some extent. (VI) The synthesis of the new cluster is completed, and α-1,6 branches are located both in the amorphous lamellae (closed circles) and in the crystalline lamellae (open circles) of the cluster.

Relations between BE, SS and DBE

In the present model, each branch-formation process is followed by the chain-elongation process. Consequently, the balance of activities between BE and SS or among BE, SS and DBE is very important in maintaining the amylopectin cluster. The fine structure of amylopectin in terms of chain-length profile alters in accord with the varied level of BEIIb activity, by using gene dosage strategy (Nishi et al. 2001) or by introducing the genomic gene encoding BEIIb into *ae* mutant line of rice (Tanaka et al. unpublished.). In fact, when the BEIIb activity is much higher than that of the wild type, normal amylopectin is replaced by water-soluble, highly branched polyglucans (Tanaka et al. unpublished). This alteration is considered to be due to the excessively high level of BEIIb activity relative to levels of SSIIa and DBE activities.

It is considered that in the *ae* mutants of Japonica rice variety, the capacity of BEIIb is inferior to that of SSIIa. When BEIIb is missing in an *ae* mutant, the number of branched chains in the crystal lamellae decreases, and almost all of the chains in the cluster are fully elongated (Fig. 2D). The model can also explain why the ratio of the number of chains within a cluster (A + B_1 chains) to that of cluster-connecting chains (B_2 + B_3 chains and longer chains) is smaller in the *ae* mutant than in the wild-type Japonica variety (Fig. 2A, D). The fact that the peak chain lengths of long chains (DP of around 40–43) are unaltered when activity of BEIIb or SSIIa is reduced (Fig. 2A, B) suggests a constant size of a cluster even in the absence of BEIIb and/or SSIIa because the skeleton of the cluster can be synthesized through branch formation in the amorphous lamellae and chain elongation by the other BE and SS isoforms. The idea is consistent with the present model.

Improper branches should be scavenged promptly and timely since they will be difficult to remove once a distorted pseudo-cluster is formed, as described above. In this sense, balance between BE and DBE activities should be maintained so that both enzymes match the rate of the cluster formation.

Analyses of effects of reduction or enhancement of the activities of all BE, SS and DBE isoforms on the fine structure of amylopectin are at least necessary to reveal the mechanism for its biosynthesis. At the same time, the fact that specificities of individual isoforms overlap each other to various extents might indicate remarkable features of plant BE, SS and DBE isoforms as compared with GBE and GS from bacteria and animals, and this may enable plants to maintain the defined structure of amylopectin.

Conclusions

The present paper describes a model explaining how an individual enzyme contributes to a distinct unit structure of the cluster in amylopectin. The model is applicable to the synthesis of amylopectin in rice endosperm. However, it could be basically applicable to amylopectin-synthesizing organs in general, although some species-specific variations undoubtedly exist. For example, the cereal endosperm contains two functionally distinct BE isoforms, BEIIa and BEIIb, while pea and potato plants have a single copy of the BEII-type gene. The present model shows that the amylopectin cluster is constructed by multiple combinations of branching and chain-elongation reactions. The combination makes it possible to easily and regularly increase both the number of chains and the length of the chains. In this process, preceding and/or adjacent clusters play an important role in providing newly forming clusters with some parts of their side chains. This supplying of chains does not result in the distortion of donor clusters if their shortened chains are elongated by SS (Fig. 3-V). According to this model, supply of side chains for the new cluster could not be seriously influenced by the amount of free chains such as malto-oligosaccharides, and the chains which are linked at the crystalline region could come from chains in the amorphous region. In this way, the number of chains per cluster can be easily made uniform. It is, however, stressed that more precise information is needed to understand the mechanism for amylopectin biosynthesis. At present, the following basic questions remain to be elucidated.

1. What determines the distinct lengths of the cluster? It might be possible that the capacity of SS for elongation of α-1,4-glucan chains markedly reduced when their lengths reach around the end of the cluster, especially when they form double helices. At the same time, BE can more efficiently attack α-1,4-glucan chains with increases of their chain lengths even when they are in double helices.
2. How are B_2 chains formed? It remains to be resolved whether after the completion of cluster synthesis, one of the B_1 and/or A chains within the cluster is selected to become B_2 chains for the subsequent cluster, or B_2 and B_3 chains are produced before or independently from the cluster formation.
3. What factor limits the number of chains per cluster?
4. Where are the A and B_1 chains derived from?
5. What are the mechanisms for enzymic reactions of branching, chain elongation and debranching?
6. How are the other enzymes such as a disproportionating enzyme, phosphorylase and amylase(s) involved in amylopectin biosynthesis?
7. What is the role, if any, of malto-oligosaccharides in amylopectin biosynthesis?
8. How do enzymes interact during starch synthesis?

Finally, it should be pointed out that to construct a model as a working hypothesis will be of great help for starch scientists to realize what aspects are lacking in our present understanding concerning the sophisticated system of amylopectin biosynthesis in plants and improve the model. I believe that various models will contribute to starch science by provoking scientists to discuss and think about important subjects in the starch biosynthesis system in plants.

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