JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Molecular Structure of Starches from Maize Mutants Deficient in Starch Synthase III

Fan Zhu,[†] Eric Bertoft,[§] Anna Källman,[#] Alan M. Myers,[⊥] and Koushik Seetharaman*[,§]

[†]School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

[§]Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55455, United States

[#]Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051, S-750 07 Uppsala, Sweden

[⊥]Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011, United States

ABSTRACT: Molecular structures of starches from *dull1* maize mutants deficient in starch synthase III (SSIII) with a common genetic background (W64A) were characterized and compared with the wild type. Amylose content with altered structure was higher in the nonwaxy mutants (25.4−30.2%) compared to the wild type maize (21.5%) as revealed by gel permeation chromatography. Superlong chains of the amylopectin component were found in all nonwaxy samples. Unit chain length distribution of amylopectins and their φ,β-limit dextrins (reflecting amylopectin internal structure) from *dull1* mutants were also characterized by anion-exchange chromatography after debranching. Deficiency of SSIII led to an increased amount of short chains (DP ≤36 in amylopectin), whereas the content of long chains decreased from 8.4% to between 3.1 and 3.7% in both amylopectin and φ,β-limit dextrins. Moreover, both the external and internal chain lengths decreased, suggesting a difference in their cluster structures. Whereas the molar ratio of A:B-chains was similar in all samples (1.1−1.2), some ratios of chain categories were affected by the absence of SSIII, notably the ratio of "fingerprint" A-chains to "clustered" A-chains. This study highlighted the relationship between SSIII and the internal molecular structure of maize starch.

KEYWORDS: *dull1 maize mutant, starch synthase III, starch structure, internal structure*

INTRODUCTION

Starch molecules are biopolymers of anhydroglucose units linked by α-1,4 and α-1,6 glycosidic bonds. They can be categorized into two types of glucose polymers, the linear amylose (extended by α-1,4 bonds) and branched amylopectin (branched by α-1,6). A branched amylose fraction was also reported depending on the separation methods used in the specific studies.[1,2] The branched chains in amylopectin are reported to be grouped along backbones in a systematic way,[3,4] into "clusters" consisting of "building blocks" that are more tightly branched.[5] The clusters and building blocks can be isolated by differential hydrolysis with α-amylase of *Bacillus amyloliquefaciens* and further characterized using enzymatic and chromatographic techniques.[4,6] On the basis of these studies, building blocks from amylopectin were categorized into six groups depending on the average number of chains per block.[5] In structural analogy, the chains with branches in amylose fractions are also clustered into building blocks. The composition and molecular structure of amylose and amylopectin have been shown to be critical to the understanding of the synthesis−structure−functional relationship of starch.[7] Amylopectin internal structure has been related to its biosynthesis, granular packing, and functionality of starch.[7−11] Starch internal structure thus has gained interest in recent years. On the basis of the internal structural feature, amylopectin of normal starches (nonmutants) from diverse plant species was categorized into four different groups.[12] Briefly, group 1 had the lowest amount of long B-chains, and the highest amount of short B-chains; group 4 contained the highest amount of long B-chains and the lowest amount of short B-chains, whereas groups 2 and 3 were intermediate between groups 1 and 4. However, mutations resulting in altered structure of starches may complicate the division system, and a general division criterion for all amylopectins including mutants may remain to be explored.

Amylopectin is synthesized by the soluble starch synthases (SS), starch branching enzymes (BE), and starch debranching enzymes (DBE), whereas amylose biosynthesis is catalyzed by granule-bound starch synthase I (GBSSI) encoded by the waxy gene.[13] Previous studies showed that functional interactions exist between multiple starch biosynthetic enzymes, and this has been observed in diverse plants;[14,15] that is, the loss of one or multiple biosynthetic enzymes may result in increased or decreased activity of other enzymes. Progress has been made in understanding the relationship of individual isoforms of enzymes in specific plant mutants, and the effects of these mutations on some characteristics of starch structure have been described.[13] Nevertheless, the mechanistic connections between the molecular activities of starch biosynthetic enzymes and the resultant fine structure of glucan polymers as they exist within starch granules remain largely unknown. The analysis of mutants could greatly contribute to the understanding of functions of individual isoforms of enzymes in relation to starch structure.

Received: May 14, 2013
Revised: August 18, 2013
Accepted: August 22, 2013
Published: August 22, 2013

Mutations of the maize *dull1* (*du1*) locus, which encodes SSIII, result in mature kernels with a dull, glassy, and tarnished phenotype[16] and altered endosperm starch structure, content, and properties.[17−20] Numerous alleles of *du1* are known including null mutations that cause complete loss of the encoded protein and a point mutation, *du1-Ref*, that results in a truncated protein that accumulates in mutant kernels.[15] Furthermore, SSIII possesses a long amino terminal extension of approximately 1200 amino acid residues beyond the SS catalytic domain, much of which is conserved in diverse plant and green algae species, thought to coordinate influence interaction with starch and other biosynthetic enzymes.[15,21,22] Previous analysis of the structure of amylopectin in *du1* mutants measured the linear chain length distribution of debranched polymers.[15] These data showed characteristic changes that could not be explained exclusively by a preference for a certain range of substrate chain lengths acted upon by the SS catalytic activity of SSIII. This is distinct from the effects of mutations in the other SS forms that are highly conserved in chloroplast-containing organisms, specifically SSI and SSII. To explain that result SSIII was proposed to coordinate the activities of multiple enzymes acting on the precursor polymers that assemble into mature starch granules.[15] Thus, it may be expected that the structures of clusters and building blocks, representing the branching zones in starch, would be altered by SSIII deficiency, and the exact structures of these affected branching zones remain unclear.

To further investigate the relationship between biosynthetic enzyme activities and the molecular organization of starch polymers within granules, this study undertook a fine structure analysis of the amylopectin present in maize endosperm starch granules. Two different *du1* alleles were utilized, specifically those either lacking SSIII or containing a truncated version, and these were compared to starch from a nonmutant line in a congenic inbred background (W64A). The influence of *du1* mutation on the molecular structures of amylose and amylopectin was examined, highlighting the internal structure of the latter as affected by the SSIII deficiency. The results were also discussed and compared with previous studies on amylopectins from diverse botanical origins with a view that amylopectins may be categorized on the basis of their internal molecular structure.

MATERIALS AND METHODS

Starches and Enzymes. The maize samples included one wild type (W64A), two single *dull1* mutants (*du1-Ref* and *du1-M3*), and one double *dull1-waxy* mutant (*du1-wx*). They were all from the W64A inbred genetic background, and the starches from the maize samples were the same specimens used in a previous study.[15] *Du1-Ref* is a point mutation that creates a stop codon resulting in a truncated protein, which is expressed at low level compared to the wild type. *Du1-M3* is a transposon insertion that is considered a null mutation, which produces no protein. *Du1-wx* is also a null mutation with no protein produced for SSIII and is also deficient in GBSSI. All of the *du1* mutations caused complete loss of SSIII enzyme activity. The exact locations and biochemistry of the mutations were given in a previous study.[15]

Amylopectin was isolated from the whole starch following the butanol−isoamyl alcohol precipitation method previously described.[23] The purification procedure was repeated two more times. The component composition (measured by chromatography as described below) remained consistent after the second purification as compared to third one, indicating the complete removal of the amylose fraction.

Rabbit muscle phosphorylase *a* (EC 2.4.1.1, specific activity = 22 U/mg) was from Sigma (St. Louis, MO, USA). *β*-Amylase of barley (EC 3.2.1.2, specific activity = 705 U/mg), isoamylase of *Pseudomonas amyloderamosa* (EC 3.2.1.68, specific activity = 210 U/mg), and pullulanase of *Klebsiella pneumoniae* (EC 3.2.1.41, specific activity = 699 U/mg) were from Megazyme (Wicklow, Ireland). The given enzyme activities were according to the suppliers.

Production of *φ*,*β*-Limit Dextrins (*φ*,*β*-LDs) from Amylopectin. Amylopectins were transformed into their *φ*,*β*-LDs by successive treatments with phosphorylase *a* (two times) and *β*-amylase (two times) as described previously.[24] Maltose, glucose 1-phosphate, and salt ions were removed by tangential flow filtration (TFF) in a Minimate TFF Capsule containing Omega 10K membrane (Pall Life Sciences, Ann Arbor, MI, USA) as described in Kong et al.[24] The *φ*,*β*-LDs were freeze-dried and sealed in plastic tubes until further use.

Debranching of Starch, Amylopectin, and *φ*,*β*-Limit Dextrins. Whole starch, amylopectin, or *φ*,*β*-limit dextrins (2 mg) were dissolved in 90% DMSO (50 μL) by gentle heating on a water bath (80 °C) and then stirring for 2 days at room temperature to completely dissolve the starch. Then hot water (400 μL, ∼80 °C) was added to dilute the sample, and the solution was allowed to cool before NaOAc buffer (0.01 M, pH 5.5, 50 μL), isoamylase (2 μL), and pullulanase (2 μL) were added. The reaction was terminated by boiling for 10 min after incubation for 24 h at room temperature. The debranched samples were analyzed by gel permeation chromatography (GPC) or high-performance anion-exchange chromatography (HPAEC) as described below.

Production and Debranching of *β*-Limit Dextrins (*β*-LDs) of Whole Starch. Whole starch (10 mg) was dissolved in 90% DMSO (150 μL) by gentle heating and then stirring for 2 days at room temperature. Then hot water (550 μL) was added to dilute the sample, and the solution was allowed to cool before NaOAc buffer (0.01 M, pH 6.0, 150 μL) and *β*-amylase (5 μL) were added. The reaction was terminated by boiling for 10 min after incubation for 24 h at room temperature. After cooling, NaOAc buffer (0.01 M, pH 5.5, 250 μL), isoamylase (2 μL), and pullulanase (2 μL) were added for debranching. The reaction was terminated by boiling for 10 min after incubation for 24 h at room temperature. The sample solutions were stored in a freezer (−20 °C) before analysis with GPC.

Gel Permeation Chromatography. Whole starch dissolved in 90% DMSO was analyzed on a column (1.6 × 45 cm) of Sepharose CL 2B (Pharmacia, Uppsala, Sweden). The injection volume was 500 μL with a carbohydrate content of ∼2.0 mg, and the eluent was NaOH (0.01 M) with a pumping speed of 0.5 mL/min. Every second fraction (0.5 mL) was analyzed for carbohydrate content by using the phenol−sulfuric acid method.[25] Remaining fractions were used to analyze λ_{max} of the iodine−glucan complex following a previous description.[23] To 0.5 mL of sample were added 1 mL of HCl (0.01 M) and 50 μL of iodine solution (0.5 g of I_2 and 0.05 g of KI/100 mL of water). Absorbance was recorded between 450 and 800 nm.

Debranched whole starch or amylopectin (∼2.0 mg/0.5 mL) was analyzed on a column (1.6 × 90 cm) of Sepharose CL 6B (Pharmacia). The eluent was NaOH (0.5 M) with a pumping speed of 1.0 mL/min. Every second fraction (1.0 mL) was analyzed for carbohydrate content by using the phenol−sulfuric acid method.[25]

High-Performance Anion-Exchange Chromatography. The unit chain distribution of debranched amylopectin and *φ*,*β*-LDs of amylopectin was analyzed by HPAEC with pulsed amperometric detection (PAD) on a Dionex ICS 3000 instrument (Sunnyvale, CA, USA). The analytical column was a CarboPac PA-100 anion-exchange column (4 × 250 mm), which was combined with a CarboPac PA-100 guard column (4 × 50 mm). The flow rate was 1.0 mL/min, and the injection volume was 25 μL with a carbohydrate concentration of 1 mg/mL. The eluent phase consisted of eluents A (0.15 M NaOH) and B (0.15 M NaOH containing 0.50 M NaOAc) using the following gradient: 0−9 min, 15−36% eluent B; 9−18 min, 36−45% B; 18−110 min, 45−100% B. The column was equilibrated with 15% eluent B for 60 min between runs. The PAD signal was converted to carbohydrate content.[26]

Statistical Analysis. Tests were conducted in triplicate and analyzed using SPSS version 19.0 software (IBM Corp., USA).

Differences between means of data were compared by least significant difference at a significance level $p < 0.05$.

RESULTS AND DISCUSSION

Composition of Amylose and Amylopectin in Starches from *dull1* Maize Mutants. The molecular size distribution obtained by GPC on Sepharose CL 2B of the starch components in normal maize starch (W64A) is compared with the *du1* mutants in Figure 1. On the basis of

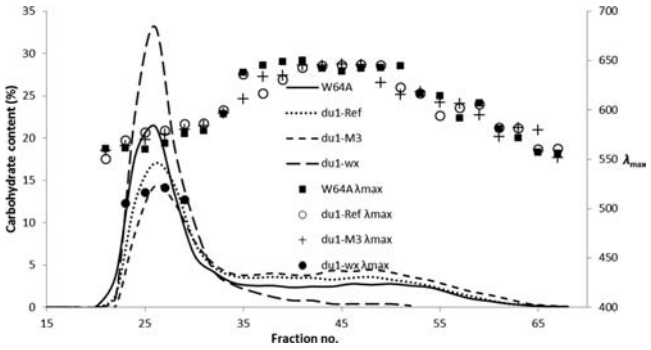

Figure 1. Fractionation of whole starches on Sepharose CL 2B.

the λ_{max} value, the material that eluted at the void volume corresponded largely to amylopectin in the nonwaxy samples ($\lambda_{max} \sim 560$ nm). In the *du1-wx* sample, however, the λ_{max} was lower (~520 nm), which suggested either that the structure of the amylopectin component was different in this sample or that some other component coeluted within the amylopectin fraction. The material that penetrated the gel possessed λ_{max} typical for amylose (~645 nm). This apparent amylose fraction was 32.9, 41.9, and 51.2% in W64A, *du1-Ref* and *du1-M3*, respectively. However, in the waxy sample there was still 10.1% of carbohydrates eluting in the region of amylose but with λ_{max} typical of its amylopectin (Table 1). The purified amylopectin fraction from the three amylose-containing samples also eluted mostly at the void volume, but a small part penetrated the gel, and this fraction was slightly larger in the *du1* samples than in W64A (Figure 2). The λ_{max} was, however, low and similar to the λ_{max} of the high-molecular-weight fraction and also similar to the apparent amylopectin fraction of the whole starches, which suggested that the material was not amylose.

Apparent amylose content was also determined from debranched whole starch analyzed by GPC on Sepharose CL 6B (Figure 3 and Table 1). According to the size, apparent amylose can be further categorized into long chains (LC_{AAM})

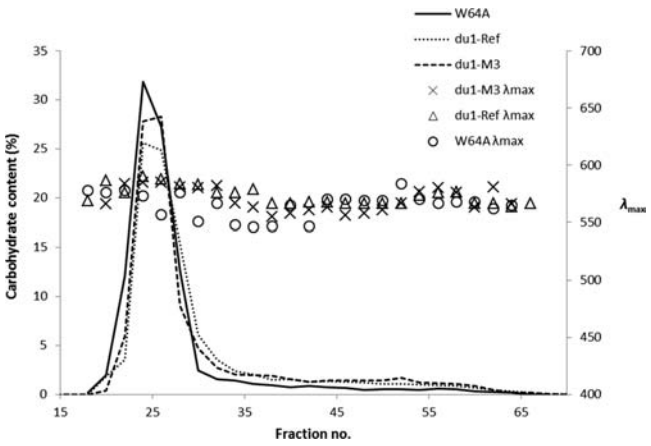

Figure 2. Fractionation of purified amylopectins on Sepharose CL 2B.

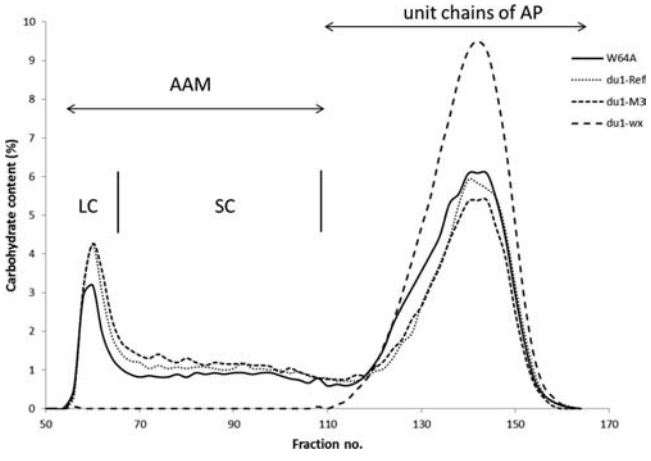

Figure 3. Fractionation of debranched whole starches on Sepharose CL 6B.

eluting around the void volume on Sepharose CL 6B (fractions 50–66) and short chains of amylose (SC_{AAM}) eluting between LC_{AAM} and amylopectin chains (fractions 68–110) (Figure 3). LC_{AAM} ranged from 11.0% (W64A) to 15.5% (*du1-M3*) and SC_{AAM} from 18.5% (W64A) to 25.0% (*du1-M3*). The composition of apparent amylose in *du1-Ref* and *du1-M3* (LC_{AAM}:SC_{AAM} = 0.62) was quite similar to the wild type (W64A) (LC_{AAM}:SC_{AAM} = 0.59). Only trace amounts of amylose in the form of LC_{AAM} (0.8%) were observed in the *du1-wx* sample, which therefore was considered as pure amylopectin. The apparent amylose contents in W64A, *du1-*

Table 1. Composition of Amylose in Starches from Maize *dull1* Mutants of the W64A Inbred Line[a]

	apparent amylose (wt %)					true amylose (wt %)				
genotype	AAM_{2B}	AAM	LC_{AAM}	SC_{AAM}	LC_{AAM}:SC_{AAM}	TAM	LC_{TAM}	SC_{TAM}	LC_{TAM}:SC_{TAM}	λ_{max} (nm)
W64A	32.9 c	29.6 c	11.0 c	18.5 c	0.59 a	21.5 c	9.8 c	11.6 c	0.84 b	645 a
du1-Ref	41.9 b	36.5 b	13.9 b	22.6 b	0.62 a	25.4 b	12.0 b	13.4 b	0.90 a	646 a
du1-M3	51.2 a	40.5 a	15.5 a	25.0 a	0.62 a	30.2 a	14.4 a	15.8 a	0.91 a	648 a
du1-wx	10.1 d	0.8 d	0.8	0	0	0.8 d	0	0	0	

[a]Different letters within a column indicate significant differences ($p < 0.05$). AAM_{2B}, apparent amylose content obtained by analysis of whole starch on Sepharose CL 2B; AAM, apparent amylose content obtained by analysis of debranched starch on Sepharose CL 6B; LC_{AAM}, long chains of apparent amylose; SC_{AAM}, short chains of apparent amylose; TAM (%), true amylose content (TAM) calculated from the difference of apparent amylose (AAM) obtained by debranching whole starch and analysis on Sepharose CL 6B and superlong chains of debranched purified amylopectin; LC_{TAM}, long chains of true amylose. SC_{TAM}, short chains of true amylose; λ_{max}, wavelength of maximum absorbance of the glucan–iodine complex obtained in fractions from GPC on Sepharose CL 2B.

dx.doi.org/10.1021/jf402090f | J. Agric. Food Chem. 2013, 61, 9899–9907

Ref, and *du1-M3* were 29.6, 36.5, and 40.5%, respectively. These values were very different from those estimated by GPC on Sepharose CL 2B of the whole starch before debranching (Table 1). Apparent amylose content obtained from Sepharose CL 2B (AAM_{2B}) was considerably higher than that from Sepharose CL 6B, clearly showing that the methods for determining the amylose content should be carefully compared (i.e., debranched starch on CL 6B vs whole starch on CL 2B).

Whole starch was subjected to β-amylolysis to obtain the β-LDs, which were further debranched and also analyzed on Sepharose CL 6B (Figure 4). An apparent amylose fraction

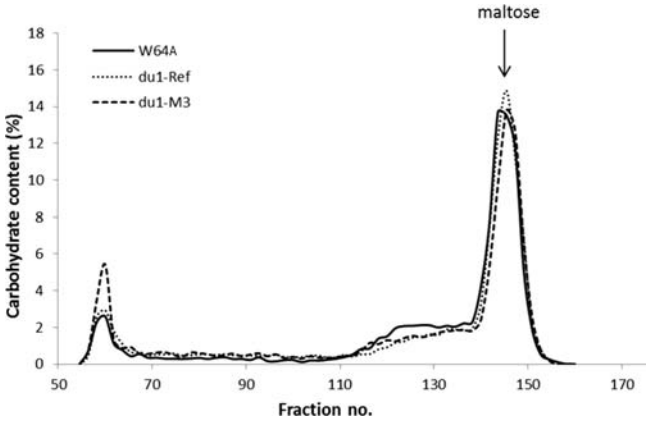

Figure 4. Fractionation of debranched β-limit dextrins of whole starches on Sepharose CL 6B.

remained in the β-LDs of the starch and possibly reflected the existence of branch points in the amylose component. The profiles suggested different branching patterns of amylose not only in the mutants compared to the wild type but also between the mutants. This further suggested that the different mutations resulting in the loss of SSIII activities may affect the activity levels of other enzymes related to starch biosynthesis to different extents, which was previously shown in maize[27,28] and also other plants.[14,29] The exact molecular mechanisms behind the interplay of the enzymes remain, however, to be explored.

Debranched pure amylopectin was analyzed on Sepharose CL 6B and suggested the existence of superlong amylopectin chains (SLC_{AP}) (Figure 5). These chains were not present in the waxy mutant (Figure 3) and explained the lower λ_{max} in the latter sample (Figure 1). The composition of SLC_{AP} is

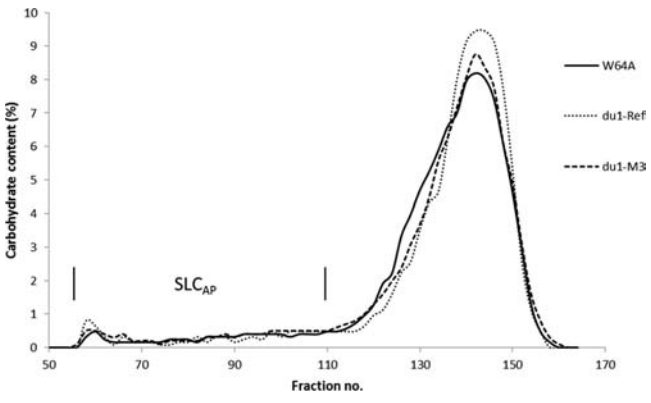

Figure 5. Fractionation of debranched purified amylopectins on Sepharose CL 6B.

presented in Table 2 wherein the *du1* mutant samples had higher amounts (10.3−11.1%) of SLC_{AP} than W64A (8.1%).

Table 2. Composition (Weight Percent) of Superlong Chains in Amylopectins from Maize *dull1* Mutants of the W64A Inbred Line[a]

genotype	SLC_{AP}	$SLC_{AP\text{-}L}$	$SLC_{AP\text{-}S}$	$SLC_{AP\text{-}L}$: $SLC_{AP\text{-}S}$
W64A	8.1 b	1.2 b	6.9 b	0.17 b
du1-Ref	11.1 a	2.5 a	8.6 a	0.29 a
du1-M3	10.3 a	1.6 b	8.7 a	0.18 b

[a]Different letters within a column indicate significant differences ($p < 0.05$). $SLC_{AP\text{-}L}$, long chains of superlong chains in amylopectins; $SLC_{AP\text{-}S}$, short chains of superlong chains in amylopectins; $SLC_{AP\text{-}L}$: $SLC_{AP\text{-}S}$, ratio of long to short chains of superlong chains in amylopectins.

The superlong chains were divided into long (eluting at the void in fractions 50−66) and short chains (fractions 68−110) eluting between the void and the majority of the amylopectin chains as indicated in Figure 5. The short chains of SLC_{AP} ($SLC_{AP\text{-}S}$) dominated the SLC_{AP} by weight (8.6−8.7% for the *dull1* samples and 6.9% for W64A) as reflected by the ratio $SLC_{AP\text{-}L}$: $SLC_{AP\text{-}S}$ (0.17−0.29). Superlong amylopectin unit chains were also found in some other starches, for example, in rice amylopectin from mutants deficient in SSIII[29] and in maize amylopectin studied by Takeda and Preiss,[30] which also had the same genetic background (W64A) as used in this work. It appeared that the increased amount of amylose content was positively correlated to that of SLC_{AP}. Indeed, GBSSI, which is encoded by the *Wx* gene and is responsible for the biosynthesis of amylose, was shown to be also responsible for the biosynthesis of superlong chains of amylopectin in rice.[31] This also explained why the waxy mutant (*du1-wx*), lacking GBSSI, had no SLC_{AP}. The existence of SLC_{AP} further suggested that at least a part of the apparent branched amylose fraction (Figure 3) could in fact be due to SLC_{AP}.

The composition of true amylose was determined on the basis of the difference between apparent amylose in the debranched whole starch and superlong chains from the debranched amylopectin.[29] Thus, true amylose contents (TAM) for W64A, *du1-Ref*, and *du1-M3* were 21.5, 25.4, and 30.2%, respectively (Table 1). It was also shown earlier that the *dull1* mutation caused increased amylose contents in starches from maize genotypes with different genetic backgrounds, including W64A inbred line,[17,19] and also from starches of other botanical species.[14,29] This clearly showed that deficiency in SSIII affected the activities of other enzymes related to biosynthesis in the starch granules.

In analogy to the division of SLC_{AP}, true amylose on Sepharose CL 6B was also categorized into long chains (LC_{TAM}) eluting at the void volume and short chains (SC_{TAM}) eluting between LC_{TAM} and amylopectin chains. LC_{TAM} ranged from 9.8% (W64A) to 14.4% (*du1-M3*) and SC_{TAM} from 11.6% (W64A) to 15.8% (*du1-M3*). The relative composition of true amylose in the mutants (*du1-Ref* and *du1-M3*) was rather similar (LC_{TAM}:SC_{TAM} = 0.90−0.91) and slightly higher than in the wild type (W64A) (LC_{TAM}:SC_{TAM} = 0.84). The ratio was considerably higher than that of the corresponding ratio of SLC in amylopectin, which showed their different nature and further supported that the long-chain fraction in the amylopectin preparation was not due to contaminating amylose.

Composition of Chain Categories in Amylopectins from *dull1* Maize Mutants. The debranched amylopectins were analyzed by HPAEC for unit chain length distributions (Figure 6 and Table 3). The unit chains of amylopectin were

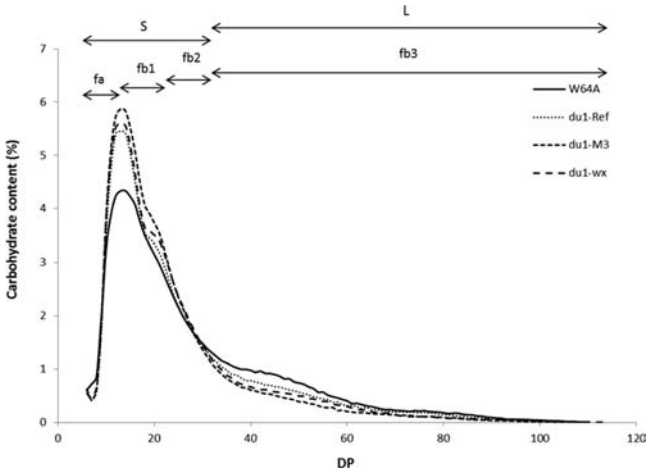

Figure 6. Fractionation by HPAEC of unit chains of debranched amylopectins.

Table 3. Chain Length Distribution (Weight Percent) of Amylopectins from Maize *dull1* Mutants of the W64A Inbred Line[a]

genotype	fa	fb1	fb2	fb3
W64A	15.5 b	41.3 c	17.9 a	24.1 a
du1-Ref	17.1 a	46.0 b	17.1 a	18.9 b
du1-M3	18.3 a	50.8 a	16.8 a	14.1 c
du1-wx	17.9 a	47.9 b	17.5 a	15.9 c

[a]Different letters within a column indicate significant differences ($p < 0.05$). The range of DP (degree of polymerization) for different fractions: fa, DP 6−12; fb1, DP 13−24; fb2, DP 25−36; fb3, DP >36.

divided into four fractions by Hanashiro et al.: fa with DP 6−12; fb1 with DP 13−24; fb2 with DP 25−36; and fb3 with DP >36.[32] These fractions have been significantly related to functional properties of various starches.[9] All *dull1* mutant samples had higher amounts of fa (17.1−18.3%) and fb1 (46.0−50.8%) fractions and lower amounts of fb2 (16.8−17.5%) and fb3 (14.1−18.9%) compared with W64A (fa, 15.5%; fb1, 41.3%; fb2, 17.9%; and fb3, 24.1%). The effect of SSIII deficiency on the molecular structure of amylopectin agreed with the previous studies on starches from diverse plant species.[10,14,15,29,33]

From a structural point of view, however, the unit chain profile is more appropriately divided into short (S) and long (L) chains at DP ∼36 (Figure 6). In this context, the long chains are thought to be B2- and B3-chains[34] (chains carrying other chains as defined by Peat et al.,[35]), whereas short chains are mixtures of A-chains (not carrying other chains) or short B1-chains. The relative amount of long chains at DP >36 was much lower in the *du1* mutants, and thus the short chains with a major peak observed at DP 13 (DP 12 on the molar basis) were more abundant. Indeed, SSIII is mainly responsible for the synthesis of longer unit chains in amylopectin.[15,29]

The external part of the amylopectin was removed by phosphorylase *a* and then *β*-amylase to obtain their *φ*,*β*-LDs.[36] By debranching of *φ*,*β*-LDs, all of the A-chains, which are completely external, appeared as maltose, and the rest were B-chains. *Dull1* mutant samples showed clearly a different internal profile in the molar distribution of diverse chain categories compared with that of the wild type (Figure 7). The molar amounts of A-chains in all samples were rather similar (52.3−54.5%) (Table 4), and it was higher than previously reported for waxy maize starch.[12] A-chains were previously divided into two subgroups:[37] Clustered A-chains (A_{cltr}-chains) were proposed to be the A-chains that participate in the formation of clusters in amylopectin, and very short A-chains in amylopectin at DP 6−8 were termed fingerprint A-chains (A_{fp}-chains), because the profile of A_{fp}-chains was shown to be fingerprinting the plant species from which the starch derives.[9,12] In this study, the profile of A_{fp}-chains from W64A (Figure 6) was consistent with a previous study on maize amylopectin.[12] In contrast, that of the *dull1* mutant samples was altered in the absence of SSIII. A_{fp}-chains, although maybe also part of the clusters,[4] could be too short to form the double helix[38] and might therefore disturb the helix formation. A_{fp}-chains are quantified from unit chain profiles of the amylopectin, whereas A_{cltr}-chains can be inferred from the difference in the amount of total A-chains and A_{fp}-chains. W64A had a higher molar amount of A_{fp}-chains (6.4%) compared with the *dull1* mutant samples (4.3−4.5%, Table 4). Instead, the relative amount of A_{cltr}-chains in the mutant samples (47.3−49.3%) was higher than in W64A (46.7%), suggesting structural differences in their clusters.[4,6]

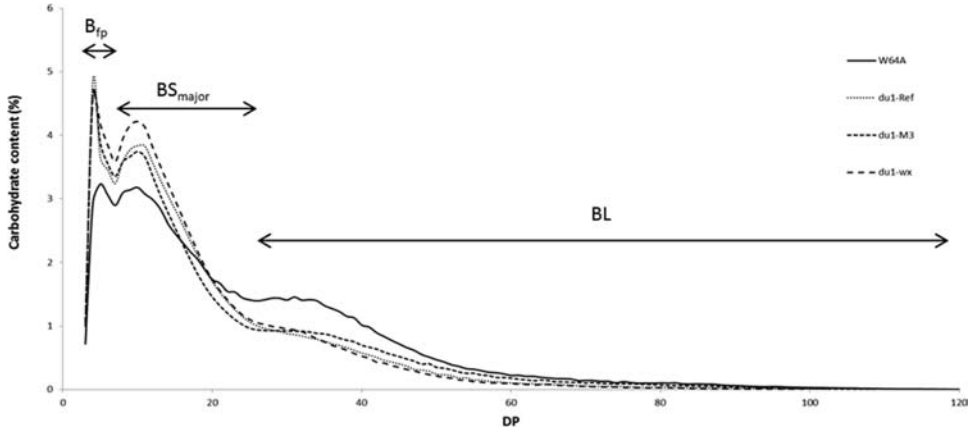

Figure 7. Fractionation by HPAEC of internal unit chains from debranched *φ*,*β*-limit dextrins of amylopectins.

Table 4. Relative Molar Content (Percent) of Diverse Chain Categories in Maize Amylopectins and Their φ,β-Limit Dextrins from Maize *dull1* Mutants of the W64A Inbred Line[a]

genotype	A	DP3	B	A_{fp}	A_{cltr}	B_{fp}	BS_{major}	BL
W64A	53.1 a	1.9 a	46.9 a	6.4 a	46.7 c	19.8 b	18.7 c	8.4 a
du1-Ref	53.6 a	2.1 a	46.4 ab	4.3 b	49.3 a	21.1 a	21.6 ab	3.7 b
du1-M3	54.5 a	2.0 a	45.5 b	4.3 b	47.3 bc	21.3 a	20.5 b	3.7 b
du1-wx	52.3 a	2.1 a	47.7 a	4.5 b	47.8 b	21.8 a	22.8 a	3.1 b

[a]Different letters within a column indicate significant differences ($p < 0.05$). A_{fp}, fingerprint A-chains of amylopectins (DP 6−8); A_{cltr}, proposed A-chains clustered in amylopectins = all A-chains − A_{fp}; B_{fp}, fingerprint B-chains of φ,β-limit dextrins (DP 3−7); BS_{major}, majority of short B-chains in φ,β-limit dextrins (DP 8−25); BL, DP ≥26−55 chains in φ,β-limit dextrins.

Table 5. φ,β-Limit Value and Chain Lengths of Chain Categories in Maize Amylopectins and Their φ,β-Limit Dextrins from Maize *dull1* Mutants of the W64A Inbred Line[a]

genotype	CL_{AP}	SCL_{AP}	LCL_{AP}	CL_{LD}	BS-CL_{LD}	BL-CL_{LD}	TICL	ECL	ICL	φ,β-LV (%)
W64A	19.7 a	15.7 c	51.4 a	8.1 a	9.6 a	38.8 a	12.6 a	13.1 a	5.6 a	58.8 a
du1-Ref	18.7 b	16.7 a	50.8 b	7.7 b	9.1 b	37.5 b	11.1 b	12.5 b	5.2 b	58.8 a
du1-M3	18.0 c	16.1 bc	50.6 b	7.6 b	9.5 a	37.7 b	11.3 b	11.9c	5.1 b	57.7 b
du1-wx	18.2 bc	16.0 bc	50.7 b	7.8 b	9.0 b	36.0 c	10.6 c	11.9 c	5.3 b	57.1 b

[a]Different letters within a column indicate significant differences ($p < 0.05$). CL_{AP}, average chain length of amylopectin; SCL_{AP}, average chain length of short chains (DP 6−36) of amylopectins; LCL_{AP}, average chain length of long chains (DP >36) of amylopectins; CL_{LD}, average chain length of φ,β-limit dextrins; BS-CL_{LD}, average chain length of short B-chains (DP 3−25) of φ,β-limit dextrins; BL-CL_{LD}, average chain length of long B-chains (DP >25) of φ,β-limit dextrins; TICL, average total internal chain length of amylopectin; ECL, average external chain length of amylopectin = CL_{AP} − CL_{LD} + 1.5; ICL, average internal chain length of amylopectin = CL_{AP} − ECL − 1, in which 1 represents the branching point; φ,β-LV, φ,β-limit value = 100 − 100 × CL_{LD}/CL_{AP}.

Unit chain of DP 3 in the φ,β-limit dextrins derives from the shortest internal segment as B-chain found in amylopectins and involves in specific structure of the clusters.[5] Previous studies observed that the amount of DP 3 dramatically increases from amylopectins to clusters as a result of α-amylolysis. All of the maize genotypes showed a rather similar amount of DP 3 (1.9−2.1%). Subcategories of B-chains are illustrated in Figure 7. Fingerprint B-chains (B_{fp}-chains, DP 3−7) were also shown to be characteristic for different samples from different plant species.[12] Indeed, the general shape of the profiles of B_{fp}-chains was consistent among the wild type and mutant samples and also agreed with a previous paper on waxy maize amylopectin.[12] However, the amount of B_{fp}-chains was higher in the *dull1* mutants (21.1−21.8%) compared to W64A (19.8%) (Table 4). Peak-DP for B_{fp}-chains was at DP 4 for the mutants and DP 5 for W64A. The majority of short B-chains (DP 8−25) was termed BS_{major}.[12] Peak-DP for BS_{major} was at DP 10 for all maize samples. As with the B_{fp}-chains, *dull1* mutant samples had higher molar amounts of BS_{major}-chains (20.5−22.8%) than W64A (18.7%). These values also showed that, in fact, B_{fp}-chains were present in practically the same number as the apparent "major" short B-chain type. Thus, with the exception for A_{fp}-chains, the molar amount of all other categories of short chains was lower in W64A than in *dull1* mutant samples and suggested possible differences in their cluster sizes and structures.

The long B-chains (BL-chains at DP >25) were suggested to be involved in the interconnections of clusters, especially in the backbone of amylopectin.[39] The longer B-chains can be categorized into B2 (DP 26−55) and B3 (DP >55) as suggested by the chain populations observed in internal profiles of many amylopectins.[12] In the maize samples in this study and in a previous paper,[12] the B3-chain population, however, was hardly distinguishable (Figure 7). The molar amounts of BL-chains were considerably higher in W64A (8.4%) than that in the *dull1* maize mutant samples (3.1−3.7%, Table 4). This suggested structural differences in the backbone structure.[4] The influence of deficiency in SSIII on the internal chain profiles of amylopectin thus resembled that of the unit chain profile of the whole amylopectin, that is, the amount of shorter chains increased while the longer chains decreased. This agreed with previous studies on the functional role of SSIII on the amylopectin structure.[10,14] It is, however, interesting to compare the *du1* maize samples with samples from *amo1* mutant barley, which also is deficient in SSIII.[40] Whereas in *du1* maize, three subcategories of short chains (A_{cltr}, B_{fp}, and BS_{major}) increased and both A_{fp} and BL decreased, only B_{fp} increased and BL decreased in *amo1* barley samples.[33] This suggests different types of changes in amylopectin fine structure due to SSIII deficiency in maize and barley.

Average chain lengths of different chain categories in φ,β-LDs were calculated (Table 5). The average chain length of the φ,β-LDs (CL_{LD}) for W64A (8.1) was longer than those of *dull1* mutant samples (7.6−7.8). The total internal chain length (TICL, defined as the length of the internal B-chains without the external single glucose residue) was also longer in W64A (12.6) than in the mutant samples (10.6−11.3). This was not only due to the fact that W64A contained more of the BL-chains, but these chains were also longer (BL-CL_{LD} = 38.8) than in the mutant samples (36.0−37.7), whereas the lengths of the short B-chains (BS-CL_{LD}) were similar to each other (9.0−9.6).

From the difference in chain length of amylopectin (CL_{AP}) and its φ,β-LD, the φ,β-limit value (φ,β-LV), average external chain length (ECL), and average internal chain length (ICL) can be calculated or inferred (Table 5). All samples had similar φ,β-LV (57.1−58.8%), which was due to the fact that W64A had both longer ECL (13.1) and ICL (5.6) compared with the maize mutants (11.9−12.5 and 5.1−5.3, respectively). This particular structural phenomenon, which is common among amylopectins from diverse species,[12] was visualized as a "rubber band",[41] which as stretched results in both longer ECL and ICL, as well as TICL, but as compressed has all segments shorter.[12] It was therefore significant to find that the *amo1*

mutants of barley starches, which were described previously,[32] possessed increased φ,β-LVs compared to nonmutants. This was due to shorter ICL and TICL, whereas ECL was similar to nonmutant barleys. Thus, the *amo1* mutants of barley did not obey the "rubber band principle", whereas the apparently similar SSIII deficiency in maize did obey this common principle. Schematic drawings on these two structural patterns are presented in Figure 8. It is therefore likely that the deficiency of SSIII in different cereals may result in different structural changes.

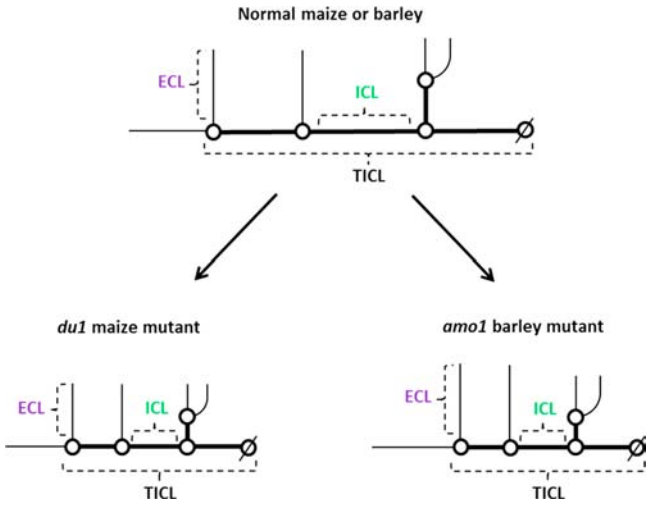

Figure 8. Effect of deficiency of SSIII on the length of chain segments in amylopectin. The proportion of external chains is approximately 60% in normal maize and barley. In the *du1* mutant of maize the proportion is similar because the length of all chain segments decreases in similar proportion. In the *amo1* mutant of barley the proportion of external chains is larger because the length of the internal chains decreases, whereas the length of the external chains remains similar to normal barley. ECL, external chain length; ICL, internal chain length; TICL, total internal chain length; thin line, external chain; thick line, internal chain; O, glucose residue involved in a branch; ⌀, reducing glucose residue.

Although the trends in the *du1* mutants compared to the control were similar, structural differences in the amylopectin were also observed between the mutant samples (Figures 6 and 7) and may be attributed to the altered levels of other enzymes related to starch biosynthesis as a result of different mutation pattern. For example, the shoulder around DP 20 (Figure 6) was more obvious and protruding in the *du1-M3* mutant compared with *du1-wx* and, especially, with *du1-Ref.* In the wild type (W64A) the shoulder was hardly noticed. Furthermore, the average CL of the whole amylopectin was slightly shorter in *du1-M3* and *du1-wx* compared to *du1-Ref* because the former possessed shorter short chains, whereas the long chains had similar lengths as in *du1-Ref* (Table 5). Also, ECL was somewhat shorter in *du1-M3* and *du1-wx*, possibly suggesting an influence on the external structure in the complete absence of the SSIII protein. However, only *du1-wx* possessed shorter TICL than the other mutants, which suggested an additional influence from the lack of GBSSI. Thus, the presence or absence of the protein, and not simply the presence or absence of the activity, seems to influence the specific structure that the amylopectin component obtains.

Molar Ratios of Diverse Chain Categories in Relation to Structural Features of Amylopectin. The molar ratio of different chain categories reflects structural features of the amylopectin component. In a previous paper, amylopectins from diverse botanical origins were categorized into four groups on the basis of their internal chain composition as reflected by molar ratios of different chain categories.[12] Previously, maize amylopectin was categorized into group 2. In this study, BS:BL, A_{cltr}:BS, and B_{fp}:BS_{major} of the wild type W64A (Table 6) fitted with the features of group 2, whereas S_{AP}:L_{AP} better fitted with that of group 3. For the *dull1* mutant samples, S_{AP}:L_{AP} instead fitted between groups 1 and 2. Also, BS:BL fitted in group 1 rather than group 2. Thus, it appeared that an absolute division into structural groups of amylopectins is not possible so far, but overlapping of the structures exists and depends on the genetic background and mutations of the plants.

Nevertheless, S_{AP}:L_{AP} may closely reflect the number of chains per cluster in amylopectin according to a previous amylopectin model.[34] Accordingly, *dull1* mutant samples, which possessed a high ratio of S_{AP}:L_{AP} (Table 6), may have more chains per cluster. The molar ratio of A- to B-chains (A:B) indicates the proportion of Staudinger[42] and Haworth[43] conformations in chain organization. All samples had similar A:B ratio (1.1−1.2), suggesting a similar portion of Staudinger and Haworth conformations. All samples also had rather similar A:BS (1.2−1.4), which apparently suggested that the composition of chains within clusters, if composed of short chains only, was unaffected by the *du1* mutation. On the other hand, the proportions of the subcategories of A-chains were very different, resulting in a much lower ratio of A_{fp}:A_{cltr} (0.09) in the mutants compared to the nonmutant (0.14). As both A_{fp}-chains and A_{cltr}-chains appeared to be part of the clusters,[4] this ratio may imply that fewer defects in the crystalline packing[44] due to incomplete double helices formation exist in the mutants. Furthermore, if only the longer "clustered" A-chains participate in double-helix formation with short B-chains,[37] the ratio of A_{cltr}:BS would be 1, which agreed well with all of the maize samples in this study. Finally, B_{fp}-chains may be found in larger building blocks that are interconnected by BS_{major}-chains within the clusters.[5] The ratio between these chains was somewhat higher in the mutants and, thus, *dull1* mutant

Table 6. Selected Molar Ratios of Diverse Chain Categories from Amylopectins and Their φ,β-Limit Dextrins from Maize *dull1* Mutants of the W64A Inbred Line[a]

genotype	S_{AP}:L_{AP}	A:B	A:BS	BS:BL	A_{fp}:A_{cltr}	A_{cltr}:B	A_{cltr}:BS	B_{fp}:BS_{major}
W64A	9.9 b	1.1 a	1.4 a	6.3 c	0.14 a	1.2 a	1.0 a	0.83 b
du1-Ref	13.6 a	1.2 a	1.3 a	9.2 ab	0.09 b	1.1 a	1.0 a	0.87 a
du1-M3	14.1 a	1.2 a	1.3 a	8.8 b	0.09 b	1.1 a	1.0 a	0.87 a
du1-wx	13.7 a	1.1 a	1.2 a	10.1 a	0.09 b	1.1 a	1.0 a	0.88 a

[a]Different letters within a column indicate significant differences ($p < 0.05$). S_{AP}:L_{AP}, molar ratio of short (DP <36) to long (DP ≥36) chains in amylopectin; BS, short chains of B-chains in φ,β-limit dextrins (BS = B_{fp} + BS_{major}); remaining diverse chain categories according to Table 4.

samples may have more large building blocks compared to W64A.

Understanding the actual organization of the unit chains in amylopectin is complicated. The results in this study suggested that differences with respect to both the external structure and the internal architecture existed between the nonmutant and the *du1* mutants, as well as within the mutant samples. Molar ratios of different chain categories in the amylopectin and its internal part may reflect the building block structure in the clusters to certain extents.[4,6] This aspect of the structure of amylopectin in the *du1* mutants will be communicated in a separate paper.

To sum up, the *du1* mutation in maize resulted in increased amylose contents in nonwaxy maize mutants and a reduction of longer chains and increase of short chains in amylopectin and their φ,β-limit dextrins, suggesting altered structure of the clusters of amylopectin. Superlong unit chains in amylopectins were found in the nonwaxy *du1* mutants and also in the wide type, but not in the waxy *du1* sample, indicating that GBSSI is responsible for their biosynthesis. The structural feature of amylopectin from the mutant samples did not fit into the same group as nonmutant maize proposed on the basis of internal structure of amylopectins from normal starches, suggesting that the division criteria are not necessarily suitable for mutant samples with altered starch synthesis. Certain molar ratios, such as A- to B-chains in amylopectin, were not affected by SSIII deficiency, suggesting the conservative aspects in starch biosynthesis. The type of *du1* mutations (*du1-Ref* vs *du1-M3*) influenced certain features of starch structure, such as amylose content and the ratio of A_{fp}:A_{cltr}-chains in amylopectin, suggesting the genetics of the mutants with the same genetic background should be carefully compared and related to the structure of starch.

AUTHOR INFORMATION

Corresponding Author

*(K.S.) E-mail: kseethar@umn.edu.

Funding

The financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Takeda, Y.; Shitaozono, T.; Hizukuri, S. Structures of subfractions of corn amylose. *Carbohydr. Res.* **1990**, *199*, 207−214.

(2) Matheson, N. K. A comparison of the structures of the fractions of normal and high-amylose pea-seed starches prepared by precipitation with concanavalin A. *Carbohydr. Res.* **1990**, *199*, 195−205.

(3) Bertoft, E. Composition of clusters and their arrangement in potato amylopectin. *Carbohydr. Polym.* **2007**, *68*, 433−446.

(4) Bertoft, E.; Koch, K.; Åman, P. Building block organisation of clusters in amylopectin from different structural types. *Int. J. Biol. Macromol.* **2012**, *50*, 1212−1223.

(5) Bertoft, E. Composition of building blocks in clusters from potato amylopectin. *Carbohydr. Polym.* **2007**, *70*, 123−136.

(6) Bertoft, E.; Koch, K.; Åman, P. Structure of building blocks in amylopectins. *Carbohydr. Res.* **2012**, *361*, 105−113.

(7) Buléon, A.; Colonna, P.; Planchot, V.; Ball, S. Starch granules: structure and biosynthesis. *Int. J. Biol. Macromol.* **1998**, *23*, 85−112.

(8) O'Sullivan, A. C.; Pérez, S. The relationship between internal chain length of amylopectin and crystallinity in starch. *Biopolymers* **1999**, *50*, 381−390.

(9) Zhu, F.; Corke, H.; Bertoft, E. Amylopectin internal molecular structure in relation to physical properties of sweetpotato starch. *Carbohydr. Polym.* **2011**, *84*, 907−918.

(10) Hanashiro, I.; Higuchi, T.; Aihara, S.; Nakamura, Y.; Fujita, N. Structures of starches from rice mutants deficient in the starch synthase isozyme SSI or SSIIIa. *Biomacromolecules* **2011**, *12*, 1621−1628.

(11) Vamadevan, V.; Bertoft, E.; Seetharaman, K. On the importance of organization of glucan chains on thermal properties of starch. *Carbohydr. Polym.* **2013**, *92*, 1653−1659.

(12) Bertoft, E.; Piyachomkwan, K.; Chatakanonda, P.; Sriroth, K. Internal unit chain composition in amylopectins. *Carbohydr. Polym.* **2008**, *74*, 527−543.

(13) Keeling, P. L.; Myers, A. M. Biochemistry and genetics of starch synthesis. *Annu. Rev. Food Sci. Technol.* **2010**, *1*, 271−303.

(14) Szydlowski, N.; Ragel, P.; Hennen-Bierwagen, T. A.; Planchot, V.; Myers, A. M.; Mérida, A.; d'Hulst, C.; Wattebled, F. Integrated functions among multiple starch synthases determine both amylopectin chain length and branch linkage location in *Arabidopsis* leaf starch. *J. Exp. Bot.* **2011**, *62*, 4547−4559.

(15) Lin, Q.; Huang, B.; Zhang, M.; Zhang, X.; Rivenbark, J.; Lappe, R. L.; James, M. G.; Myers, A. M.; Hennen-Bierwagen, T. A. Functional interactions between starch synthase III and isoamylase-type starch-debranching enzyme in maize endosperm. *Plant Physiol.* **2012**, *158*, 679−692.

(16) Mangelsdorf, P. C. The inheritance of amylaceous sugary endosperm and its derivatives in maize. *Genetics* **1947**, *32*, 448−458.

(17) Boyer, C. D.; Liu, K. C. The interaction of endosperm genotype and genetic background. Part 1. Differences in chromatographic profiles of starches from nonmutant and mutant endosperms. *Starch/Staerke* **1985**, *37*, 73−79.

(18) Li, J.; Corke, H. Physicochemical properties of maize starches expressing dull and sugary-2 mutants in different genetic backgrounds. *J. Agric. Food Chem.* **1999**, *47*, 4939−4943.

(19) Wang, Y. J.; White, P.; Pollak, L.; Jane, J. Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. *Cereal Chem.* **1993**, *70*, 171−179.

(20) Wang, Y. J.; White, P.; Pollak, L.; Jane, J. Amylopectin and intermediate materials in starches from mutant genotypes of the Oh43 inbred line. *Cereal Chem.* **1993**, *70*, 521−525.

(21) Gao, M.; Wanat, J.; Stinard, P. S.; James, M. G.; Myers, A. M. Characterization of *dull1*, a maize gene coding for a novel starch synthase. *Plant Cell* **1998**, *10*, 399−412.

(22) Palopoli, N.; Busi, M. V.; Fornasari, M. S.; Gomez-Casati, D.; Ugalde, R.; Parisi, G. Starch-synthase III family encodes a tandem of three, starch-binding domains. *Proteins: Struct., Funct., Bioinf.* **2006**, *65*, 27−31.

(23) Klucinec, J. D.; Thompson, D. B. Fractionation of high-amylose maize starches by differential alcohol precipitation and chromatography of the fractions. *Cereal Chem.* **1998**, *75*, 887−896.

(24) Kong, X.; Corke, H.; Bertoft, E. Fine structure characterization of amylopectins from grain amaranth starch. *Carbohydr. Res.* **2009**, *344*, 1701−1708.

(25) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.

(26) Koch, K.; Andersson, R.; Åman, P. Quantitative analysis of amylopectin unit chains by means of high-performance anion-exchange chromatography with pulsed amperometric detection. *J. Chromatogr., A* **1998**, *800*, 199−206.

(27) Cao, H.; Imparl-Radosevich, J.; Guan, H.; Keeling, P. L.; James, M. G.; Myers, A. M. Identification of the soluble starch synthase activities of maize endosperm. *Plant Physiol.* **1999**, *120*, 205−215.

(28) Singletary, G. W.; Banisadr, W.; Keeling, P. L. Influence of gene dosage on carbohydrate synthesis and enzymatic activities in

endosperm of starch-deficient mutants of maize. *Plant Physiol.* **1997**, *113*, 293−304.
(29) Fujita, N.; Yoshida, M.; Kondo, T.; Saito, K.; Utsumi, Y.; Tokunaga, T.; Nishi, A.; Satoh, H.; Park, J. H.; Jane, J. L.; Miyao, A.; Hirochika, H.; Nakamura, Y. Characterization of SSIIIa-deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the rice endosperm. *Plant Physiol.* **2007**, *144*, 2009−2023.
(30) Takeda, Y.; Preiss, J. Structures of B90 (sugary) and W64A (normal) maize starches. *Carbohydr. Res.* **1993**, *240*, 265−275.
(31) Hanashiro, I.; Itoh, K.; Kuratomi, Y.; Yamazaki, M.; Igarashi, T.; Matsugasako, J. I.; Takeda, Y. Granule-bound starch synthase I is responsible for biosynthesis of extra-long unit chains of amylopectin in rice. *Plant Cell Physiol.* **2008**, *49*, 925−933.
(32) Hanashiro, I.; Abe, J. I.; Hizukuri, S. A periodic distribution of chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydr. Res.* **1996**, *283*, 151−159.
(33) Bertoft, E.; Källman, A.; Koch, K.; Andersson, R.; Åman, P. The cluster structure of barley amylopectins of different genetic backgrounds. *Int. J. Biol. Macromol.* **2011**, *49*, 441−453.
(34) Hizukuri, S. Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydr. Res.* **1986**, *147*, 342−347.
(35) Peat, S.; Whelan, W. J.; Thomas, G. J. Evidence of multiple branching in waxy maize starch. *J. Chem. Soc., Chem. Commun.* **1952**, 4546−4548.
(36) Bertoft, E. Partial characterization of amylopectin α-dextrins. *Carbohydr. Res.* **1989**, *189*, 181−193.
(37) Bertoft, E. On the nature of categories of chains in amylopectin and their connection to the super helix model. *Carbohydr. Polym.* **2004**, *57*, 211−224.
(38) Gidley, M. J.; Bulpin, P. V. Crystallisation of maltooligosaccharides as models of the crystalline forms of starch: minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.* **1987**, *161*, 291−300.
(39) Källman, A.; Bertoft, E.; Koch, K.; Åman, P.; Andersson, R. On the interconnection of clusters and building blocks in barley amylopectin. *Int. J. Biol. Macromol.* **2013**, *55*, 75−82.
(40) Li, Z.; Li, D.; Du, X.; Wang, H.; Larroque, O.; Jenkins, C. L. D.; Jobling, S. A.; Morell, M. K. The barley amo1 locus is tightly linked to the starch synthase IIIa gene and negatively regulates expression of granule-bound starch synthetic genes. *J. Exp. Bot.* **2011**, *62*, 5217−5231.
(41) Wikman, J.; Larsen, F. H.; Motawia, M. S.; Blennow, A.; Bertoft, E. Phosphate esters in amylopectin clusters of potato tuber starch. *Int. J. Biol. Macromol.* **2011**, *48*, 639−649.
(42) Staudinger, H.; Husemann, E. Über hochpolymere Verbindungen. 150. Mitteilung. Über die Konstitution der Stärke. *Liebigs Ann. Chem.* **1937**, *527*, 195−236.
(43) Haworth, W. N.; Hirst, E. L.; Isherwood, F. A. Polysaccharides. Part XXIII. Determination of the chain length of glycogen. *J. Chem. Soc., Chem. Commun.* **1937**, 577−581.
(44) Genkina, N. K.; Wikman, J.; Bertoft, E.; Yuryev, V. P. Effects of structural imperfection on gelatinization characteristics of amylopectin starches with A and B-type crystallinity. *Biomacromolecules* **2007**, *8*, 2329−2335.