A simplified method of determining the internal structure of amylopectin from barley starch without amylopectin isolation

Xue Zhao a, *, Mariette Andersson b, Roger Andersson a

a Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-750 07, Uppsala, Sweden
b Department of Plant Breeding, Swedish University of Agricultural Sciences, Box 101, SE-230 53, Alnarp, Sweden

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ABSTRACT

To determine the internal structure of barley starch without amylopectin isolation, whole starch was hydrolyzed using β-amylase to remove the linear amylose and obtain β-limit dextrins (β-LDs). The β-LDs were treated with extensive α-amylase to prepare α-limit dextrins (α-LDs), and the α-LDs were further hydrolyzed with β-amylase into building blocks. The chain-length distribution of β-LD and building block composition were analyzed by size-exclusion chromatography and anion-exchange chromatography. The internal structure of the barley whole starches had similar pattern to barley amylopectins analyzed by conventional methods. The starch of barley amo1-mutated varieties contained more short internal B-chains and less long internal B-chains than that of other varieties. The starch from amo1-mutated varieties had more large building blocks than that from waxy varieties. The simplified method presented in this study can effectively characterize starch internal structure that relates to physicochemical properties of starch, although some details of amylopectin structure are not assessable.

1. Introduction

Normal starch consists of amylose and amylopectin, both of which are polysaccharides built up of 1,4-linked α-glucose monomers. Amylose is basically a long linear molecule, while amylopectin is a highly branched molecule that contains thousands of chains of glucose monomers connected through 1,6-linkages. The chains in amylopectin can be classified into three types (Pear, Whelan, & Thomas, 1952): A-chains (carrying no other chains), B-chains (carrying A- and/or other B-chains), and C-chains (carrying the reducing end). The internal part of amylopectin can be studied by removal of the external chains using an exo-acting enzyme like β-amylase, resulting in a β-limit dextrin (β-LD) with all branch points intact and A-chains degraded into maltosyl or maltotriosyl stubs (Bertoft, 2004). Internal chain-length distribution, can then be studied by debranching β-LDs using isoamylase and pullulanase. A previous study on the structure of whole starch and β-LD in barley categorized B-chains as: fingerprint B-chains (Bfp-chains) with degree of polymerization (DP) 4–7; short B-chains (BS) including Bfp-chains and BSmajor-chains with DP 8–27 (subdivided into DP 8–14 and DP 15–27); and long B-chains (BL) with DP ≥ 28 (Källman, Bertoft et al., 2015).

Building blocks, likely one of the most informative aspects of starch structure, are the basic internal structural units and have a major effect on the physical properties of starch (Källman, Vamadevan et al., 2015). Building blocks can be categorized into five different groups based on size, the largest containing approximately 10–12 chains per block, on average, and the smallest (most common) containing two chains per block (Bertoft, Koch, & Åman, 2012; Bertoft, Koch, & Åman, 2012). The distance between branch points of the building blocks ranges from DP 1–3 (Bertoft et al., 2012b). Building blocks with tight branching patterns are conventionally obtained by partial hydrolysis with endo-acting α-amylase from Bacillus amyloliquefaciens, followed by successive extensive hydrolysis with phosphorylase and β-amylase (Bertoft, Källman, Koch, Åman, & Åman, 2011; Bertoft, Källman, Koch, Andersson, & Åman, 2011).

Studies on starch internal structure are important in order to understand the biosynthesis of starch and the relationship between starch structure and properties. Overall, most of the methods used to date for investigating amylopectin structure involve first isolating amylopectin from whole starch and then hydrolyzing the amylopectin molecule with α-amylase into domains, clusters, and building blocks step-by-step (Bertoft et al., 2011a, 2011b, 2012a, 2012b; Källman, Bertoft, Koch, Åman, & Andersson, 2013). However, the amylopectin isolation and partial α-amylolysis steps are usually time-consuming and
labor-intensive, and require relatively large amounts of sample (grams) to obtain enough material to characterize structures at the level of building blocks (Lemos, Barbosa, Ramos, Coelho, & Družnik, 2019; Zhu, Bertof, & Seetharaman, 2013). Moreover, it has been shown that α-amylolysis of amylopectin or of its β-LD (i.e. the sequences of enzymes α-amylase + β-amylase or β-amylase + α-amylase) practically give the same result (Bertof, 1989). Zhu et al. (2013) have attempted to reduce the working load of analyzing internal structure of maize starch without prior amylolysis isolation. However, the whole starch was still hydrolyzed by partial α-amylolysis to obtain the clusters first, and then the clusters were subsequently treated with β-amylase and α-amylase to obtain building blocks. Branches from the amylopectin fraction may only comprise around 1–2% of total branches in whole starch (Zhu et al., 2013). Thus in normal starches, branched amylose fractions might not influence the results when studying the structure of building blocks of starch. However, the internal chain-length distributions in the amylopectin fraction may be slightly different, due to the increased amylose content, when studying de-branched β-LDs of whole starch. Examining the structural features of whole starch might therefore provide a broader picture, and an alternative viewpoint for understanding the relationships between structure-synthesis and structure-function.

The aim of this study was to develop a simplified analytical method for effective characterization of starch internal structure (mainly internal chain-length distribution and building block composition) without prior amylolysis and amylopectin separation. The method should be possible to be easily applied for different kinds of starch samples even when sample amount is very limited. The structure information obtained by the method could be used to predict some functional properties, such as retrogradation of starch from different botanical sources and varied genetic backgrounds. The method was tested using barley whole starches analyzed by conventional methods in a previous study (Källman, Vamadevan et al., 2015). These barley starches have different mutations, resulting in varied levels of amylose content and different molecular structure to study.

2. Materials and methods

2.1. Starches and enzymes

One waxy barley and one waxy potato pure starch were provided by Lyckeby Starch AB (Kristianstad, Sweden). An additional 10 barley starch samples (Cinnamon (waxy), SW28708 (waxy), Cindy (waxy), NGB 114602 (normal starch), Gustav (normal starch), KVL 301 (normal starch, low β-glucan), SLU 7 (normal starch, high β-glucan), SW 49427 (amol1 and wax mutant), Glacier Ac38 (amol1 mutant), and Karmose (possessing the amol1 mutation)) were obtained as starch. The starch from the barley cultivars Cinnamon, Cindy, Glacier Ac38 and SW 49427 was isolated and purified at Lyckeby Starch AB (Kristianstad, Sweden) according to Andersson, Andersson, and Åman (2001). The starch from the other 6 barley samples was isolated and purified in a previous study (Källman, Vamadevan et al., 2015). These same samples had been used in previous studies of amylopectin structure (Bertof et al., 2011a, 2011b) and starch properties (Källman, Vamadevan et al., 2015) by conventional methods.

α-Amylase from B. amylophilus varicis ([1,4]-α-α-glucan glucoamylase; E-BAASS, EC 3.2.1.1, specific activity ~50 U/mg), β-amylase of barley ([1,4]-α-α-glucan maltodextrinase; E-BARBL, EC 3.2.1.2, specific activity ~400 U/mg), isoamylase (glycogen 6-glucanohydrolase; E-ISAMYHP, EC 3.2.1.68, specific activity ~240 U/mg), and pullulanase M1 (Klebsiella planticola) (pullulan 6-α-glucanohydrolase; E-PULKP, EC 3.2.1.41, specific activity ~30 U/mg) were purchased from Megazyme (Wicklow, Ireland). Before carrying out the enzyme hydrolysis, the β-amylase, isoamylase, and pullulanase were de-salted twice using sodium acetate (NaOAc) buffer through PD-10 desalting columns (Sephadex, Amersham Pharmacia Biotech AB, Uppsala, Sweden). The manufacturing protocol of the column was exactly followed for desalting enzymes. The desalting step was necessary because these three enzymes were stored in 3.2 M ammonium sulfate, which interfered with the high-performance size-exclusion chromatogram. The enzyme (700 μL) was applied for desalting and 7 mL desalted enzyme was collected in total after the double desalting procedure. The final concentration/activity of the desalted enzymes was 10-fold dilution of the original enzymes, and the desalted enzymes were used in hydrolyses of the study within one week, except for time-course analysis of β-amylolysis.

2.2. Time-course analysis of β-amylolysis of amylopectin/whole starch

The waxy barley and waxy potato starch samples (100 mg) were dissolved in 3 mL of 90% aqueous dimethylsulfoxide (DMSO) by gently heating in a boiling water bath for 15 min with occasional vigorous stirring, and diluted with 32 mL water (4°C) denized water. Then 3.5 mL 0.01 M NaOAc buffer pH 6.0 and 200 μL barley β-amylase were added to start the reaction in a water bath (4°C) with constant magnetic stirring. An aliquot (2 mL) was taken at 0 min, 10 min, 20 min, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 24 h. These aliquots were heated in a boiling water bath for 5 min to stop the enzymatic reaction and then centrifuged for 10 min at 1000g. As a measure of the rate of β-amylolysis of starch, the maltose released was assayed. A subsample (0.25 mL) from each aliquot taken at the 11 time points was thoroughly mixed with 0.75 mL deionized water and 2.0 mL of Sumner’s reagent (Hostettler, Borel, & Deuel, 1951) containing 1% (w/v) 3,5-dinitrosalicylic acid in water (Merck, Darmstadt, Germany). The solution was heated for 5 min in a boiling water bath, then cooled in running water. Absorption was measured using a spectrophotometer (Shimadzu, Kyoto, Japan) at 530 nm. A standard curve was made using maltose monohydrate (Merck, Darmstadt, Germany) with defined concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL) of maltose solution. All samples were measured in duplicate and the concentration of reducing sugars was calculated (Fink, Andersson, Rosen, & Åman, 2006). The yield of β-LDs was calculated by subtraction of maltose residue content from 100%.

2.3. Production of β-limit dextrin (β-LD)

A sample of starch (12.5 mg) was dissolved in 0.875 mL of 90% aqueous dimethylsulfoxide (DMSO) by gently heating in a boiling water bath for 15 min with occasional vigorous stirring, and diluted with 2 mL warm deionized water. Then 0.875 mL 0.01 M NaOAc buffer, pH 6.0, and 250 μL barley β-amylase (diluted 10-fold and desalted twice using 0.01 M NaOAc buffer, pH 6.0) were added to start the β-amylolysis. The samples were left for 1 h in a water bath (40°C) with constant magnetic stirring (based on results from the time-course analysis) and the reaction was terminated in a boiling water bath for 5 min. After cooling to room temperature (around 25°C), 16 mL absolute ethanol (99.5%) were added to 4 mL of the β-amylolysate mixture and the solution was stored in a refrigerator (4°C) for 1 h to precipitate the β-LDs by centrifugation for 15 min at 1000g (Fig. 1).

2.4. Production of α-limit dextrin (α-LD)

Hot deionized water (0.9 mL) was added to dissolve the precipitated β-LDs (about 5 mg), with the help of scraping and stirring. Then 0.1 mL 0.01 M NaOAc buffer, pH 6.5, and 45 μL α-amylase were added for extensive α-amylolysis overnight (18 h) in a water bath (40°C) with constant magnetic stirring (Fig. 1). The final α-amylolysis concentration was about 6 U/mL. The reaction was terminated in a boiling water bath for 5 min.

2.5. Production and characterisation of building blocks

To ensure that no external chains remained in the resulting building blocks (Fig. 1), 200 μL 0.01 M NaOAc buffer, pH 6.0, and 300 μL barley β-amylase (diluted 10-fold and desalted twice using 0.01 M NaOAc buffer, pH 6.0, and 200 μL barley β-amylase were added to start the reaction in a water bath (40°C) with constant magnetic stirring. An aliquot (2 mL) was taken at 0 min, 10 min, 20 min, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 24 h. These aliquots were heated in a boiling water bath for 5 min to stop the enzymatic reaction and then centrifuged for 10 min at 1000g. As a measure of the rate of β-amylolysis of starch, the maltose released was assayed. A subsample (0.25 mL) from each aliquot taken at the 11 time points was thoroughly mixed with 0.75 mL deionized water and 2.0 mL of Sumner’s reagent (Hostettler, Borel, & Deuel, 1951) containing 1% (w/v) 3,5-dinitrosalicylic acid in water (Merck, Darmstadt, Germany). The solution was heated for 5 min in a boiling water bath, then cooled in running water. Absorption was measured using a spectrophotometer (Shimadzu, Kyoto, Japan) at 530 nm. A standard curve was made using maltose monohydrate (Merck, Darmstadt, Germany) with defined concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL) of maltose solution. All samples were measured in duplicate and the concentration of reducing sugars was calculated (Fink, Andersson, Rosen, & Åman, 2006). The yield of β-LDs was calculated by subtraction of maltose residue content from 100%.
overnight (18 h) in a water bath (40 °C) were added, and the mixture was constantly stirred.-LDs (about 5 mg from step 2.3) isolated from starch, with the help of a membrane filter (0.45 μm), and analyzed by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC) (with 5- or 10-fold dilution of sample solution, if needed).

2.6. Characterization of de-branched β-LD

Hot deionized water (0.9 mL) was added to dissolve the precipitated β-LDs (about 5 mg from step 2.3) isolated from starch, with the help of scraping and stirring. Then 300 μl isomalto- and 300 μl pullulanase (both enzymes diluted 10-fold and desalted twice using 0.01 M NaOAc buffer, pH 6.0) were added for debranching overnight (18 h) in a water bath (40 °C) with constant stirring. The de-branched samples were heated in a boiling water bath for 5 min to terminate the reaction, filtered through a membrane filter (0.45 μm), and analyzed by HPSEC and HPAEC (with 5- or 10-fold dilution of sample solution, if needed).

2.7. High-performance anion-exchange chromatography (HPAEC)

An HPAEC device (Series 4500i, Dionex Corp., Sunnyvale, CA, USA) equipped with a BioLC gradient pump and a pulsed amperometric detector (PAD) was employed in this study. Separation was performed on a Carbopac PA-100 (4 × 250 mm) analytical column (Dionex, Sunnyvale USA) equipped with a guard column. Elution was performed at 25 °C, with a flow rate of 1 mL/min, and the injection volume was 25 μL, using 0.15 M NaOH (eluent A) and 0.50 M NaOAc + 0.15 M NaOH (eluent B). The de-branched β-LDs were eluted with the following gradient: 0–15 min, 15–28 % B; 15–45 min, 28–55 % B; 45–75 min, 55–70 % B; and 75–80 min 70–15% B (return to the start mixture). The column was equilibrated with 15 % eluent B for 15 min between runs. The building blocks were eluted with the following gradient: 0–20 min, 15–28 % eluent B; 20–35 min, 28–50 % B; 35–45 min, 50 % B; and 45–50 min 50–15% B (return to the start mixture). The column was equilibrated with 15 % eluent B for 15 min between runs. The PAD response of de-branched β-LDs was converted to carbohydrate concentrate (weight %) and further to molar percentage (M%) according to Koch, Andersson, and Åman (1998) and normalized by total molar weight (Table 1). The PAD response of building blocks was not converted, and is presented as relative peak area (Table 2). All samples were analyzed in duplicate and the results presented are the mean of two replicates.

2.8. High-performance size exclusion chromatography (HPSEC)

All samples were analyzed as described previously (Andersson, Fransson, Tietjen, & Åman, 2009), with minor modifications. The HPSEC is equipped with refractive index (RI) detector (Wyatt Technology Corp., Santa Barbara, CA). Two serially connected OHpak SB-802.5 HQ columns with a guard column (Shodex, Showa Denko KK, Miniato, Japan) were kept at 35 °C. The eluent was 0.1 M NaNO₃, containing 0.02 % NaN₃, with a flow rate of 0.5 mL/min. Data were analyzed using ASTRA software (version 4.7.0.07, Wyatt Technology Corp., Santa Barbara, CA). The results presented are the mean of two replicates, and the sample blank was subtracted since enzyme and buffer peaks were found between elution volume 15–16 mL and 17–18 mL (Supplementary Fig. S1). For small fragments, the columns were calibrated with glucose, maltotriose, maltotetraose, and maltopentaose. The HPAEC chromatograms were normalized to elution volume 18 mL, after which maltotriose, maltose, and glucose were eluted (Supplementary Fig. S1).

2.9. Statistical analyses

Differences in starch structure based on HPAEC results between the barley starch samples with the amo1 mutation (n = 3), those with the wax mutation (n = 3), and normal starch (n = 4) were studied by one-way analysis of variance (ANOVA) and Tukey comparisons, using Mininstab 18 (State College, PA, USA). Principal component analysis (PCA) was used to obtain an overview of starch structure in the 10 barley starch samples based on HPSEC results, using SIMCA 16.0.1 software (Umetrics, Umeå, Sweden). Pareto scaling was applied during the data handling process.

3. Results and discussion

3.1. Time-course analysis of β-amylosylation

The hydrolysis of amylopectin by β-amyrase was studied using a waxy potato and waxy barley starch, in order to determine an appropriate β-amylose treatment time. It was found to be rapid in the first half-hour and then slowed down (Fig. 2). The hydrolysis was almost complete after 1 h and only slightly more hydrolysis occurred after 24 h of incubation (Fig. 2). Therefore, 1 h of β-amylosylation was chosen in method development to obtain β-LDs. The yield of β-LDs at 1 h was found to be 53–54 %, calculated by subtracting the content of maltose residue from 100 %. This was close to the content of β-LDs reported in the literature (59–60 % for barley starch, 56 % for potato starch) (Hizukuri, Abe, & Hanashiro, 2015).

3.2. Internal chain-length distribution

The internal chain-length distribution of whole starch (without amylose and amylopectin separation) from the 10 barley varieties was investigated by β-amylosylation followed by debranching the β-LDs with isoamyase and pullulanase and subsequent analysis with HPAEC and HPSEC, respectively. Maltose and maltotriose were excluded from the HPAEC results in calculation of molar proportion, since maltose and a
4

Table 1

Composition of chain-length categories of β-limit dextrins (β-LDs) with degree of polymerization (DP) 4–50, based on relative molar composition analyzed by HPAEC, in barley starches with the amo1 mutation (n = 3), starches with the wax mutation (n = 3), and normal starches (n = 4).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Characteristics</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
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<tbody>
<tr>
<td>Cinnamon</td>
<td>Waxy</td>
<td>54.3</td>
<td>27.0</td>
<td>10.2</td>
<td>4.6</td>
<td>3.9</td>
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<td>SW28708</td>
<td>Waxy</td>
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<td>27.3</td>
<td>10.0</td>
<td>4.4</td>
<td>3.6</td>
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<tr>
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<td>26.6</td>
<td>10.3</td>
<td>4.6</td>
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<td>26.1</td>
<td>10.0</td>
<td>4.8</td>
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<tr>
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<td>26.6</td>
<td>9.9</td>
<td>4.3</td>
<td>3.8</td>
</tr>
<tr>
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<td>10.5</td>
<td>4.9</td>
<td>4.6</td>
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<td>5.1</td>
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<td>25.2</td>
<td>10.2</td>
<td>5.0</td>
<td>5.4</td>
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<tr>
<td>Waxy starch</td>
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<tr>
<td>Normal starch</td>
<td>54.1± 26.5± 10.3±</td>
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<td>Amo1 starch</td>
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Mean ± standard deviation are shown for the three starch types, with different superscript letters indicating statistically significant differences.

Chains were divided as: B_0 = DP 4–7; BS_maj−chains were sub-divided into DP 8–14 and DP 15–27; long B-chains (BL) = DP ≥ 28.

Table 2

Composition of building blocks in five different size groups (G2-G6), based on relative peak area analyzed by HPAEC, in barley starch samples with the amo1 mutation (n = 3), wax mutation (n = 3), and normal starch (n = 4).

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<td>10.8</td>
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<tr>
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<td>Amo1, waxy</td>
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<td>27.4</td>
<td>11.5</td>
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Mean ± standard deviation are shown for the three starch types, with different superscript letters indicating statistically significant differences.

Fig. 2. Time-course analysis of β-amylolysis of waxy barley and waxy potato starch samples. The standard deviations of the replicates were lower than 1.7.

major part of maltotriose originate from A-chains, with only a minor part of maltotriose originating from B-chains (Bertoft, 2004). Thus, the molar proportion calculated in this study only showed the internal chain-length distribution of B-chains from whole starch as internal chain profile, represented by the β-LDs of whole starch.

This internal chain length distribution represented by the β-LDs of whole starch was summarized into different size categories, as shown in Table 1. Starch from the barley varieties Glacier Ac38, Karmosé, and SW49427, all possessing the amo1 mutation, had a significantly higher proportion of B0-chains (p < 0.001) and lower proportion of long chains with DP ≥ 28 (p < 0.05) than waxy and normal barley starches. The amo1 mutation barley starches also had a slightly lower proportion of short internal B-chains with DP 8–14 (p < 0.05) than the normal barley starches, and a slightly lower proportion of B0-maj−chains with DP 15–27 (p < 0.05) compared with those in the waxy barley starches studied (Table 1).

Overall, it was apparent from the HPAEC results that the internal chain length distribution of β-LDs in the whole starch samples analyzed was of two distinct types that correlated with the genetic background of the barley varieties. The three waxy barley starches contained more B0-maj−chains with DP 15–27 and internal long B-chains; whereas starch from the amo1 mutation barley lines had more ‘fingerprint’ (B0) chains (Table 1). These results confirm previous findings (Bertoft et al., 2011b; Källman, Vadamedavan et al., 2015; Matsuki, Yasui, Sasaki, Fujita, & Kitamura, 2008). It has been shown that the amo1 background is associated with a higher proportion of B0 chains and a lower proportion of long (BL) chains (Matsuki et al., 2008). Similar structural features have been found in the amylopectins of barley varieties SW49427, Glacier Ac38, and Karmosé, which contain more B0 chains and have a lower molar proportion of chains with DP ≥ 38 (Bertoft et al., 2011b; Källman, Vadamedavan et al., 2015).

However, the HPSEC results for barley starch possessing the amo1 mutation, especially the barley varieties Glacier Ac38 and Karmosé, showed a different pattern, with more very long chains which eluted between elution volume 12 mL and 13 mL (Fig. 3, Supplementary Fig. S2). The very long internal B-chains may have originated from the amylose fraction, since a previous study on potato starch using the same HPSEC settings indicated that the fraction eluted before elution volume 13 mL is the major amylose fraction (unpublished results). Compared with the waxy barley varieties, starch from the amo1 mutation lines showed more B0−chains which eluted between elution volume 16.5 mL and 18 mL and less intermediate internal B-chains which eluted between elution volume 13.7 mL and 16.5 mL (Fig. 3). Compared with Glacier Ac38 and Karmosé, the starch from SW49427 (possessing both amo1 and wax mutations) had fewer chains that eluted before elution volume 14.7 mL and almost no very long chains eluted before elution volume 13 mL from the amylose fraction (Supplementary Fig. S2). In a previous study,
starch from SW 49427 was found to have an amylose content of 3.7 %, while Glacier Ac38 and Karmosé had an amylose content of 32.6 % and 47.8 %, respectively, much higher than in the waxy barleys Cinnamon, SW 28708, and Cindy (amylose content 0.0, 0.6, and 10.2 %, respectively) (Källman, Vamadevan et al., 2015). The higher amylose content is most likely the reason why Glacier Ac38 and Karmosé starch showed a higher proportion of very long chains in the HPSEC analysis. Moreover, the debranched intermediate material may also explain some variations of long internal chains among the starches, since the intermediate material can vary from 4 % to 9 % in normal and high amylose starches (Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001). However, these very long chains originating from the amylose fraction did not contribute to the internal chain-length distribution in HPAEC analysis, because they were beyond the HPSEC separation range (Table 1). In other words, the proportion of long chains in our HPAEC analysis mostly represented the long internal B-chains of amylopectin, while the long chains shown by HPSEC analysis also included the very long chains from the amylose fraction.

In the PCA plot of de-branched β-LDs analyzed with HPSEC, the three waxy barley varieties grouped in the lower part of the diagram and the three amoI mutation barley varieties in the upper part (Fig. 4). However, there was some variation between Glacier Ac38, Karmosé, and SW49427 (possessing both amoI and wax mutations). All normal barley varieties except SLU 7 were located in the central part of the score plot, whereas SLU 7 tended to group with the amoI mutants (Fig. 4 and Supplementary Fig. S2).

### 3.3. Building block distribution

In the HPAEC chromatogram, the building-block profiles of the 10 barley starch samples were similar, and only slight differences between samples were observed. In order to compare the results of our method with those of conventional analyses, we grouped the building blocks based on the pattern of peaks in the chromatograms according to the published protocols (Bertoft et al., 2011a; Zhu et al., 2013), with minor modifications. The groups were numbered G1-6, in order of increasing size of building blocks: G1 comprised linear dextrans and a minor part of the smallest building blocks with DP = 3; G2, G3, and G4 represented blocks with two chains, three chains, and four chains, respectively; and G5 and G6 contained several types of blocks, which were not distinguished as specific populations (Bertoft et al., 2011a). The small, linear dextrans in G1 are produced by α-amylase attacking the internal chain segments between building blocks, mainly resulting in maltose and large amounts of glucose and maltotriose. In addition, maltotetraose, maltopentaose, and maltohexaose are produced. On adding β-amylase, the latter dextrans were converted into DP 1–3 (Bertoft et al., 2011a; Zhu et al., 2013). Therefore, group G1 was excluded and the HPAEC building block profile of groups G2–G6 from one barley starch sample is shown as an example in Fig. 5.

It was found that the starch of varieties SW49427, Glacier Ac38, and Karmosé, with the amoI mutation, had significantly more (p < 0.05) large building blocks of G5 and G6 than the starch of Cindy, Cinnamon and SW28708, with the wax mutation. A previous study also found that the large clusters in two amoI mutation starch samples, namely Karmosé and Glacier Ac38, had more large building blocks than the other samples analyzed (Bertoft et al., 2011a). A study comparing starch from the 10 barley varieties used in the present study suggested that the starch from barley varieties possessing the amoI mutation has more large building blocks, a more compact structure, and a slightly increased degree of branching (DB), although the molar proportions of chain categories in the clusters were similar in all 10 barley starch samples (Källman, Vamadevan et al., 2015). The distribution of different sizes of building blocks within different clusters and domain categories was determined in previous studies. In contrast, the building block structure in our study was determined on the complete collection of building blocks after successive β-amylase, α-amylase, and β-amylase enzyme treatments on the whole starch samples. Overall, our results were mostly in agreement with previous findings. There was good agreement on the composition of large building blocks between barley whole starches in our study and
barley amylopectin clusters identified by conventional analysis in the previous study (Källman, Vamadevan et al., 2015) (Fig. 6). Similarly, the starch of barley varieties with the amo1 mutation was enriched in the large building blocks of G5 and G6, in agreement with previous findings (Table 2 and Fig. 6). The distribution of building blocks was related to the varieties of barley, indicating that the size distribution of the building blocks was dependent on the genetic background. In addition, SLU 7 tended to have large building blocks (Table 2 and Supplementary Fig. S3), as also found for building blocks in barley clusters in the previous study (Källman, Vamadevan et al., 2015). In that study, variety SLU 7 tended to group with the amo1 mutants, but the genetic background of this barley is not known.

The differences in building block groups between samples were also apparent from the HPSEC results, with more large building blocks and less small blocks in the starch of barley varieties SW49427, Glacier Ac38, and Karmosé (with the amo1 mutation) compared with Cindy, Cinnamon, and SW28708 (with the wax mutation) (Fig. 7). The normal barley varieties were positioned between these two genetic backgrounds (wax and amo1). These patterns of building blocks were supported by the PCA on HPSEC results, where the amo1 group was separated with the waxy group, while the normal barley starches located between the two

4. Conclusions

The internal structure (internal B-chain distribution and building block composition) of 10 whole barley starches with different genetic background and phenotype characteristics was determined using a method without amylopectin isolation and with an existing method. Internal B-chains distribution and the composition of building blocks from whole starches had very similar patterns according to both methods. With our simplified method, starches from amo1 mutants characteristically contained more large building blocks and fewer internal long B-chains, but a higher proportion of fingerprint (B fp) chains, than waxy barley starches. These parameters seemed to be correlated with genetic background and could be used to predict some functional properties, such as retrogradation of starch from different botanical
sourced and varied genetic backgrounds. However, some details of amylopectin structure like domains and clusters may not be assessable or identical to those observed when studying isolated amylopectin. The very long internal B-chains due to some branching in amylose may bring challenges or opportunities for understanding the internal B-chain distribution in HPSEC analysis. Whereas, HPAEC gave better resolution compared to HPSEC in analysis of internal B-chain distribution of amylopectin, since the de-branched β-LDs are linear chains and separate according to DP. However, the building blocks had different complex structures, even at the same DP, resulting in complex chromatograms on HPAEC. In this regard, HPSEC gave a slightly better overview of building block composition.

**Author contribution**

R.A. conceived and designed the research. X.Z. and R.A. designed the experiments. X.Z. conducted the experiments and wrote the manuscript. All authors read, edited, and approved the manuscript.

**CRediT authorship contribution statement**

Xue Zhao: Writing - original draft, Writing - review & editing. Mariette Andersson: Writing - original draft, Writing - review & editing. Roger Andersson: Writing - original draft, Writing - review & editing.

**Declaration of Competing Interest**

The authors report no declarations of interest.

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**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.carbpol.2020.117503.

**References**


