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Thermal properties of barley starch and its relation to starch characteristics

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This publication is dedicated to the memory of Dr. Koushik Seetharaman (1966–2014).

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ABSTRACT

Amylopectin fine structure and starch gelatinization and retrogradation were studied in 10 different barley cultivars/breeding lines. Clusters and building blocks were isolated from the amylopectin by α-amylase from *Bacillus amyloliquefaciens* and their structure was characterized. Gelatinization was studied at a starch:water ratio of 1:3, and retrogradation was studied on gelatinized starch at starch:water ratio of 1:2, by differential scanning calorimetry. Three barley cultivars/breeding lines possessed the *amo1* mutation, and they all had a lower molar proportion of chains of DP ≥38 and more of large building blocks. The *amo1* mutation also resulted in a higher gelatinization temperature and a broader temperature interval during gelatinization. Overall, small clusters with a dense structure resulted in a higher gelatinization temperature while retrogradation was promoted by short chains in the amylopectin and many large building blocks.

1. Introduction

Two types of starch granules are synthesized in the barley endosperm [1–4]. A-granules, which are large lenticular shaped, with a diameter of ~10–13 μm, and B-granules, which are small and spherical, with a diameter of ~3 μm [2,4,5]. By weight, A-granules constitute more than 90% of the starch [2], however, less than 20% by number [2,5]. Barley A-granules have a longer average chain length (CL) of amylopectin, higher amylose content [2,6–8], and gelatinize at a lower temperature than B-granules [6,8,9]. For these reasons, a high and representative yield of isolated starch is important when studying the correlation between genetic background, amylopectin structure and the physicochemical properties of starch.

Normal barley starch consists of 25–30% amylose and 70–75% amylopectin [10]. Both amylopectin and amylose are polysaccharides built up of 1,4-linked α-D-glucose. Amylose is basically a long linear molecule, while in amylopectin chains of glucose monomers are connected through 1,6-linkages, forming a highly branched structure. These chains are categorized into three types, namely A-, B-, and C-chains [11]. A-chains carry no other chains, B-chains can carry both A-chains and other B-chains, and the C-chain carries the sole reducing end-group. A-chains with a degree of polymerization (DP) of 6–8 are called fingerprint A-chains (A_{fp}-chains), since they have been shown to possess typical profiles depending on crop [12]. The chains in amylopectin are organized into clusters, which have been defined as groups of chains where the internal chain length (ICL), i.e. the distance between branch points, is shorter than 9 glucosyl residues [13]. The basic structural unit, however, are the building blocks, which are tightly branched units with an ICL of ~1–2 residues [14]. Five different size-groups of building blocks have been categorized, with the smallest one, group 2, consisting of only two chains, and the largest blocks consisting of at average ~10–12 chains. Barley amylopectin possessing the *amo1* mutation have previously been shown to have less long chains of DP ≥38 [15–17], more large clusters and a shorter ICL [15] as well as a higher amylose content [16–18].

The external chains of the amylopectin molecule, i.e. the part of the amylopectin chains extending from the outermost branch point, form double helices in the crystalline lamellae of the starch

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granules. Three types of X-ray patterns of the granules can appear depending on the organization of these double helices, namely A-, B- or C-type [19,20]. Cereal starch generally has the A-type pattern, while B-type pattern is found in root and tuber starch, and the C-type pattern, which is a mixture of A- and B-types, is found in legume starch [19]. Crystalline amylose inclusion complexes form a V-type pattern. The internal structure of amylopectin can be studied by removing the external chains enzymatically, keeping all branch points intact. Using β-amylase and phosphorylase *a* in sequence, a φ,β-limit dextrin is obtained, in which all A-chains are degraded into maltosyl stubs [21] and the shortest B-chain have a DP of three glucosyl residues [22]. In the limit dextrin, the shortest B-chains having a DP of 3–7 are called fingerprint B-chains (B_{fp}-chains), since their chain length distribution profile differs between crops [12,21]. The other B-chains are further categorized into B1a(l), with a DP of 8–17, B1b, of DP 18–27, and B2-chains with a DP ≥28.

In amylopectin, a higher proportion of short chains of DP 6–12 has been shown to result in a lower gelatinization temperature [23–26] and enthalpy [24,25], while a higher proportion of chains of DP 13–24 correlates with a higher gelatinization temperature [23,24,26]. Probably, short chains cause defects in amylopectin crystallites causing them to melt at a lower temperature [26,27]. Vamadevan et al. demonstrated that internal organization of amylopectin, length of the external chains and the amount of A_{fp} chains (Dp 6–8) determine the registration of double helices within the crystalline lamellae and thereby gelatinization temperature of the starches [28]. Also, the amylose/amylopectin ratio has been shown to influence gelatinization temperature [29] and retrogradation [29–31].

This investigation aimed to study the correlation between amylopectin fine structure, amylose content and thermal properties of starch from normal and mutant barleys.

2. Materials and methods

2.1. Barley samples

Barley flour used in this study was from the six varieties NGB 114602 (normal starch), high β-glucan barley SLU 7, low β-glucan barley KVL 301, waxy barley SW 28708, high amylose *amo1* mutant barley Karmosé and the normal barley Gustav. All six varieties were grown in Chile during summer 2010. The amylopectin structure of these six barley cultivars/breeding lines was investigated as well as their physical properties. Physical properties of starch were also investigated in the four barleys Cinnamon (waxy), Cindy (waxy), Glacier Ac38 (*amo1* mutant) and SW 49427 (waxy and *amo1* mutant) and were of the same batches as previously used to study amylopectin fine structure [15,32,33].

2.2. Starch isolation

Barley starch isolation was based on the method by McDonald and Stark [34]. Barley flour (10 g) was steeped in 100 mL 0.02 M HCl and the pH adjusted to between 2.5 and 3.0. The mixture was stirred for 10 min, neutralized with 0.02 M NaOH and mixed with an Ultra-Turrax T-25 (Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) for 3 min using a 10 mm rod at 9500 min^{-1}. The slurry was filtered through a 70 μm mesh cloth and the fiber residue was rubbed with mortar and pestle. Starch and fiber residue were centrifuged separately at 4000 × *g* for 20 min and the pellets were each dissolved in 50 mL 0.1 M Tris–HCl buffer (pH 7.6, containing 0.5% $NaHSO_3$ and 0.01% thiomersal). Both crude starch and fiber residue were incubated with 1 mg of proteinase K (EC 3.4.21.64, 30 U/mg protein, Sigma–Aldrich, St. Louis, MO, USA) at 25 °C for 24 h. After centrifugation at 4000 × *g* for 20 min the fibrous residue was filtered through a 70 μm mesh cloth and washed out starch was added to the previous starch fraction and centrifuged as above. The starch pellet was dispersed in 100 mL of 0.1 M Tris–HCl buffer and again incubated with 2 mg proteinase K at 25 °C for 24 h. After centrifugation at 4000 × *g* for 20 min the pellet was dissolved in 300 mL 0.2 M NaCl, mixed with 300 mL toluene and left to stir for 16 h. The sample was centrifuged as above and the toluene layer removed by suction. Any granules remaining in the toluene–aqueous interface were retrieved and washed with water. All of the starch was dispersed in 300 mL 0.2 M NaCl, mixed with 300 mL toluene and stirred for 16 h. The sample was centrifuged as above, the toluene layer removed by suction and granules remaining in the toluene-aqueous interface were retrieved and washed with water. All starch was washed twice with acetone and air-dried. All structural analyses were performed on this isolated starch. To avoid any effects of fiber, i.e. β-glucan, on the enthalpy of gelatinization [35] the starch was subjected to a second purification before DSC measurements and WAXS analysis where the starch was filtered through a 70 μm mesh cloth, centrifuged at 8200 × *g* for 10 min and the upper brown layer was removed. The brown layer and the white starch were suspended in water and the procedure was repeated several times with both fractions and the white starch fractions were pooled. The starch was finally washed twice with acetone and air-dried.

The purity of the starch isolates was analyzed in duplicate with the method by Åman et al. [36] using 50 μL of thermostable α-amylase (EC 3.2.1.1, 3000 U/mL, Megazyme, Wicklow, Ireland), 100 μL (32.6 U) amyloglucosidase in acetate buffer (EC 3.2.1.3, 3260 U/mL, Megazyme) and 3 mL GOPOD reagent (Megazyme).

2.3. Amylopectin isolation

Isolation of amylopectin was performed as described by Klucinec and Thompson [37] with minor modifications according to Bertoft et al. [15]. However, all centrifugations were done at 5000 × *g* for 30 min at 4 °C. The amylopectin fractions from Karmosé and SLU 7 were treated a second time in order to reach a high purity. The purity was analyzed by gel-permeation chromatography as described below.

2.4. Production of clusters and building blocks

Clusters were isolated from amylopectin essentially according to Bertoft et al. [15]. Amylopectin (200 mg) was dissolved in 4 mL 90% DMSO by gentle heating and diluted with 14 mL of warm water. After cooling to 25 °C, 2 mL α-amylase of *Bacillus amyloliquefaciens* (EC 3.2.1.1, Megazyme, Wicklow, Ireland) in 0.01 M NaOAc buffer, pH 6.5 was added, giving a final enzyme concentration of 0.09 U/mL. The activity of the enzyme was measured as described by Bertoft et al. [38]. The sample was incubated at 25 °C for 90 min (based on results from a time-course on waxy barley) and the reaction was stopped by addition of 400 μL 5 M NaOH and left at room temperature for 1 h. The sample was precipitated with 5 volumes of methanol at room temperature overnight, centrifuged at 3000 × *g* at 6 °C for 20 min and washed two times with ethanol. Clusters were transformed into φ,β-limit dextrins (φ,β-LDs) according to Kong et al. [39], but 5 mL of the samples was collected from the series of PD-10 columns instead of 4.5 mL.

Building blocks (Bbls) were produced from φ,β-LDs of clusters according to Kong et al. [39]. After the reaction was terminated, the following analyses were performed: (i) aliquots of 0.5 mL were analyzed by gel-permeation chromatography as described below, (ii) to 100 μL of the sample 400 μL water was added and the sample analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described by Bertoft [13]; (iii) to 100 μL sample, 300 μL water and 100 μL 0.01 M NaOAc buffer, pH 7.2, and 4 μL pullulanase M1 from *Klebsiella*