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Characterization of internal structure of maize starch without amylose and amylopectin separation

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ABSTRACT

Normal maize starch was used to characterize the internal structure of starch without separating amylose and amylopectin, and the result was compared with amylose-free waxy maize starch. The clusters in the whole starch were produced by partial hydrolysis using α-amylase of *Bacillus amyloliquefaciens*, and were subsequently treated with β-amylase to remove the linear amylose and to produce β-limit dextrins of clusters from amylopectin. The clusters were further hydrolyzed extensively with α-amylase to produce building blocks. The compositions of clusters in the form of β-limit dextrins and their building blocks were analyzed by gel-permeation chromatography and high-performance anion-exchange chromatography. The results showed that the structures of clusters and building blocks from whole starch of normal and waxy starches were similar. By number, each cluster contained 9–10 chains and 5–6 building blocks. The inter-block chain length in the clusters of whole starch was around six glucosyl residues. This study explored an alternate procedure to characterize the composition of branches in whole starch without separating amylose and amylopectin components.

1. Introduction

Starch as biopolymers of glucose may be regarded as a mixture and spectrum of structures with various molecular sizes (elongated by α-(1→4) bonds), degree of branching (by α-(1→6) bonds), average chain-lengths and number of chains (Matheson, 1990). Separation of starch components has been attempted by ultracentrifugation, gel-permeation chromatography, selective aqueous leaching, retrogradation, preferential precipitation with iodine or alcohol, and complexing with concanavalin A (Matheson, 1990). Among these methods, butanol precipitation of starch components (Schoch, 1942) is still most commonly employed. Butanol-precipitated fraction (amylose) contains some branches, which, however, are few by number compared with the non-precipitated fraction (amylopectin) (Takeda, Hizukuri, Takeda, & Suzuki, 1987; Takeda, Shitaozono, & Hizukuri, 1990). Depending on the separation methods and varied conditions of the same method, the amounts and branching density of branched amylose in the amylose fraction varied (Matheson & Welsh, 1988; Matheson, 1990; Vilaplana, Hasjim, & Gilbert, 2012). A method for separation of essentially linear amylose from slightly branched amylose remains to be developed (Buléon, Colonna, Planchot, & Ball, 1998), and the nature of the branched amylose in relation to specific separation methods used is still to be explored. Calculation based on previous studies (Takeda et al., 1987; Takeda, Shitaozono, & Hizukuri, 1988) showed that the branches from amylose fractions (isolated from butanol precipitation based method) may contribute to around 1–2% of the total branches in the whole starch. Overall, the methods used so far suggest that the amount of branched amylose fractions in normal starches is small compared to amylopectin fractions.

In many scenarios in research, starch from biological organisms like leaves or algae (Ball, Colleoni, Cenci, Raj, & Tirtiaux, 2011; Smith, 2012) or from breeding lines are only available in very small quantities (in milligrams) for structural characterization. Traditional ways of separating amylose and amylopectin, such as with butanol precipitation, is time and labour intensive, and usually requires relatively large amounts of sample (in grams) to get enough fractions to finally analyze structures at the building block levels. This limits the possibilities to explore structural features of interesting mutants or other research questions related to starch synthesis. A method of using whole starch to obtain a full structural analysis on starch branching patterns remains to be developed. Furthermore, in the broader picture, understanding structural features of whole starch might provide an alternative viewpoint of structure–synthesis and structure–function relationships.

α-Amylase of *Bacillus amyloliquefaciens* (Priest, Goodfellow, Shute, & Berkeley, 1987) has been a powerful tool to characterize the cluster and building block structure of amylopectin (Bertoft, 1990, 2007a, 2007b). α-Amylase of *B. amyloliquefaciens* has a higher propensity to act as an endo-acting enzyme compared to other

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α-amylases (Goesaert, Bijttebier, & Delcour, 2010). Thus it can be used to efficiently release clusters from starch with limited hydrolysis (Bertoft, 1990). The released clusters in the form of φ,β-limit dextrins (φ,β-LDs) or β-LDs can be structurally elucidated by diverse chromatographic techniques. The average size of the clusters from starches from diverse plant origins reported hitherto ranges from DP 50 to 90 and the number of chains per cluster ranges from 8 to 16 (Bertoft, Koch, & Åman, 2012a). When the clusters are further subjected to more extensive hydrolysis by α-amylase, building blocks with smaller size and tighter branching patterns than clusters are obtained (Bertoft, Koch, & Åman, 2012b). These building blocks are practically α-LDs. The internal chain length of the building blocks is between DP 1 and 3. The most common building blocks are of the smallest size (DP 5–9) and contain 2 chains per block.

Internal structure of starch molecules (mostly amylopectin) has been shown to be related to the biosynthesis of starch molecules, organization and packing of the granules, and eventually the functional properties for applications (Buléon et al., 1998; O'Sullivan, & Pérez, 1999; Vamadevan, Bertoft, & Seetharaman, 2013; Zhu, Corke, & Bertoft, 2011). Thus, the structure and organization of clusters and building blocks, which are the structural elements of the internal parts of the biomacromolecules, is likely one of the most informative aspects of starch structure.

In this study, whole normal maize starch from the endosperm was used for characterization of the structure of clusters and building blocks without separating amylose from amylopectin, and the result was compared with waxy maize starch (containing no amylose). The result reported here is a first step towards the development of small-scaled analytical methods for starch internal structural characterization that can be used in studies related to starches from diverse tissues, such as leaves and pericarp, when the samples are available in limited amounts.

2. Materials and methods

2.1. Starches and enzymes

Normal maize starch (Melojel) was obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). The amylose content was 22.8% determined after debranching and gel-permeation chromatography (Sargeant, 1982). Waxy maize starch was from AVEBE, Belgium.

α-Amylase of *B. amyloliquefaciens* (EC 3.2.1.1), 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 activity of the α-amylase (413 U/mL at pH 6.5, 25 °C) was determined based on a previous description (Bertoft, Manelius, & Qin, 1993).

2.2. Time course analysis of α-amylolysis for cluster production from whole starch

Whole starch (20 mg) was dissolved in 400 μL of 90% dimethyl sulfoxide (DMSO) by heating in boiling water bath and then constant stirring for two days at room temperature (~21 °C). Hot double-distilled water (1.4 mL) was then added to the sample. After cooling to 25 °C, 200 μL of α-amylase (0.9 U/mL) in NaOAc buffer (0.01 M, pH 6.5) was added to start the reaction in a water bath with magnetic stirring. The concentrations for substrate and α-amylase in the reaction system were 10 mg/mL and 0.09 U/mL, respectively. Samples (200 μL) were taken from 10 min up to 150 min. If not analyzed immediately, 4 μL of NaOH solution (5.0 M) was added and mixed to destroy the α-amylase, and then stored at −18 °C. If analyzed immediately, the sample was diluted in 200 μL water and 40 μL of NaOH solution (5.0 M), and analyzed by GPC on Sepharose CL 6B as described below.

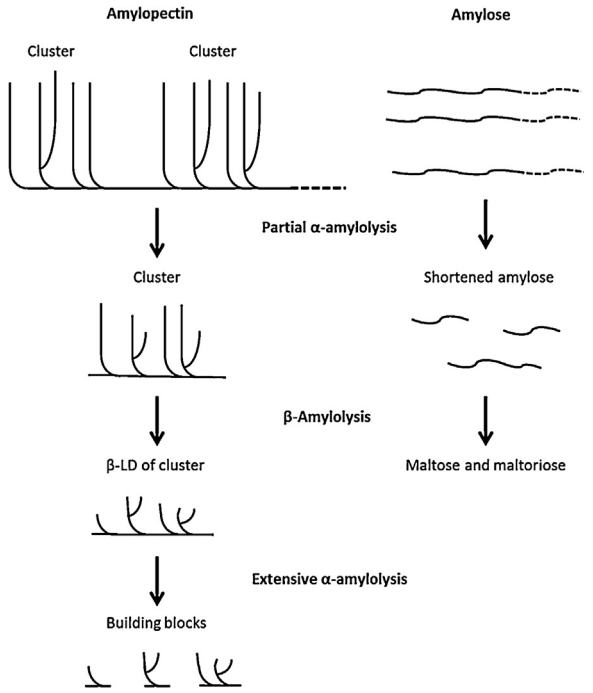

Fig. 1. Fate of amylose and amylopectin in the course of differential hydrolysis by α-amylase of *B. amyloliquefaciens* for cluster and building block production.

2.3. Production of clusters

Whole starch (80 mg) was dissolved in 1.6 mL of 90% dimethyl sulfoxide (DMSO) by gentle heating and then constant stirring for two days at room temperature. Hot double-distilled water (5.6 mL) was added and, after cooling to 25 °C, 800 μL of α-amylase (0.9 U/mL) in NaOAc buffer (0.01 M, pH 6.5) was added to start the reaction in a water bath (25 °C) with magnetic stirring. The reaction was continued for 100 min before being stopped by adding 160 μL NaOH (5.0 M) and standing at room temperature for 1 h to destroy the α-amylase. Pure methanol (40 mL) was added to precipitate the clusters in the form of α-dextrins. The sample was left at 4 °C for 3 h before the precipitate was recovered by centrifugation (4000 × *g*, 20 min) at room temperature. The precipitate was washed twice with pure methanol (10 mL) and let stand in fume hood overnight to remove the methanol through evaporation. The principle of the methods and fate of starch components are illustrated in Fig. 1. Amylose component is illustrated as linear since the number of branches in amylose fractions is rather limited compared with that of amylopectin as discussed in Section 1.

The α-LDs of clusters were re-dissolved in hot water and the carbohydrate content was adjusted to 10 mg/mL. NaOAc buffer (0.33 volumes, 0.01 M, pH 6.0) and β-amylase (3 μL) was added at 40 °C. The reaction continued for 3 h and was stopped by boiling for 10 min.

The clusters in the form of β-limit dextrins were separated from maltose and the buffer salt on two PD-10 columns (GE Healthcare Life Sciences, NJ, USA) coupled in tandem (Kong, Corke, & Bertoft, 2009). The filtration through the PD-10 columns was done twice before the clusters were freeze-dried.