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Amylolysis of amylopectin and amylose isolated from wheat, triticale, corn and barley starches

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ABSTRACT

Amylopectin (AP) and amylose (AM) were isolated from normal (wheat, triticale, corn, and barley), waxy (corn and barley), and high-amylose (corn and barley) starches. The relationship between the molecular characteristics of the above starch polymers and amylolysis was studied in comparison to their native granules. Amylolysis was conducted by using granular starch hydrolyzing enzymes (a mixture of α-amylase and glucoamylase) at sub-gelatinization temperatures (<55 °C) over a period of 1 to 72 h, and the degree of hydrolysis (DH) was measured as a percentage of reducing value. At the early stages (1 h) of hydrolysis, AP and AM fractions from all starches were significantly hydrolyzed by amylases to a greater extent than native granules. Regardless of starch sources, the DH of AP and AM at 1 h hydrolysis ranged from 71.4 to 86.1 and 66.4 to 81.4%, respectively. Between 1 and 72 h of hydrolysis, the DH of AP was higher than AM in normal and high-amylose starches. Significant correlations were found between the molecular weight (M_w) or molecular size (R_z) of AP, and the DH of native granules at 1 h hydrolysis. The results suggested that a high proportion of short-chains in AP, indicated by its high M_w, high R_z and low average chain-length, were responsible for high DH of native granules. The difference in DH among native granules was mainly influenced by the difference in granular architecture resulting from variations in average chain length of AP.

1. Introduction

Starch is the second abundant natural polysaccharide present in higher plants, next to cellulose and it is an inevitable source of energy for animals, including humans. In green plants, starch is deposited as granules and the architecture of a starch granule is built up by two polymers of glucose, essentially a linear amylose (AM) and a heavily branched amylopectin (AP), which are highly organized through intra- and inter-molecular hydrogen bonds resulting in a complex biopolymer.

In North America, cereal starches are more frequently used in various food and industrial applications than starches from other sources. One of the current food trends is the consumption of starchy products that are rich in resistant starches (RS). RS can be used as a functional food ingredient for making variety of food products, since some of these can be a source of dietary fiber (i.e. completely resistant to enzyme hydrolysis) and others are known as slowly digestible starches, demonstrated to assist in controlling sugar metabolism in diabetics (Englyst, Kingman, & Cummings, 1992; Liu, 2005; Mason, 2009). On the other hand, starches are traditionally being used for bioethanol (a renewable source of energy) production (Gomez, Steele-King, & McQueen-Mason, 2008) and in North America, the bioethanol production mainly relies on starches from corn and wheat grains. The ethanol production requires starch granules from grains to be enzymatically hydrolyzed completely to sugars (glucose, maltose and maltotriose), which are subsequently fermented to ethanol by yeast (Chen, Wu, & Fukuda, 2008; Sharma, Rausch, Tumbleson, & Singh, 2007).

Despite the extensive collection of starch hydrolysis (by amylases) studies reported in the literature, a substantial research gap yet exists in this area of science. There is no information available on how AM and AP would be hydrolyzed by amylases when isolated from the starch granule. A comparison of the reactivity of amylases towards the intact native granule and its isolated components (AM and AP) would help scientists to understand the role played by AM and AP in starch amylolysis.

In general, enzymatic hydrolysis is used as a tool to study the architecture of starch granules (Miao, Zhang, Mu, & Jiang, 2011). Hydrolysis of starch granules with amylases (i.e. amylolysis) occurs in several steps, which include diffusion to the solid surface,

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adsorption, and finally catalysis. The rate of hydrolysis is initially fast but then continues at a slower and more persistent rate (Oates, 1997). Among the amylases, α-amylase and glucoamylase are most commonly used to study the hydrolysis pattern of starch granules. The α-amylases (EC 3.2.1.1) are endo-acting enzymes that internally hydrolyze α-D-(1,4)-glycosidic linkages of both AP and AM yielding soluble products such as oligosaccharides, and branched and low molecular weight α-limit dextrins. However, glucoamylase (EC 3.2.1.3) is an exo-acting enzyme, which depolymerizes both α-(1,4)- and α-(1,6)-linkages of starch polymers from their non-reducing ends resulting in the complete conversion of starch into glucose (Sujka & Jamroz, 2007). Thus, a study of the action pattern of amylases is important for understanding the impact of starch structure on physicochemical properties and functionality. The influence of structural properties of native starches such as granule size, granule architecture, and granule porosity on *in vitro* hydrolysis have been studied (Asare et al., 2011; Dhital, Shrestha, & Gidley, 2010; Liu, Gu, Donner, Tetlow, & Emes, 2007; Naguleswaran, Li, Vasanthan, Bressler, & Hoover, 2012; Naguleswaran, Vasanthan, Hoover, & Bressler, 2013; Salman et al., 2009; Stevnebø, Sahlström, & Svihus, 2006; Sujka & Jamroz, 2007; Uthumporn, Zaidul, & Karim, 2010). However, there is a dearth of information on the extent to which isolated AM and AP are hydrolyzed by α-amylase and glucoamylase.

The molecular structural features of AM and AP influence the starch hydrolysis. The molecular characteristics of AP such as molar mass (molecular weight), molecular dimension or size (radius of gyration), molecular density, branching degree, and distribution of short-chains have been shown to influence starch hydrolysis by amylases (Goesaert, Bijttebier, & Delcour, 2010; Miao et al., 2011; Murthy, Johnston, Rausch, Tumbleson, & Singh, 2011). For instance, AP molecules with higher number of short-chains with a greater degree of branching (resulting in more compact structure/high molecular density, high molar mass and small molecular size of AP molecule) are less susceptible to hydrolysis by amylases (i.e. a property preferred in low-glycemic food production). In contrast, starch granules composed of AP molecules with low molecular density and molar mass could be useful for bioethanol production, as it requires rapid but a complete hydrolysis by amylases. Thus, the objective of this study was to compare the reactivity of amylases towards the intact native granule and isolated AP and AM in order to understand the role played by molecular characteristics of AP and AM on starch amylolysis.

2. Materials and methods

2.1. Materials

Two cultivars of wheat grains, Canada prairie spring red (CPS Red) and AC Reed, were provided by Alberta Agriculture and Food in Barrhead (AB, Canada). Triticale grains (Pronghorn and AC Ultima) were obtained from the Field Crop Development Centre of Alberta Agriculture and Rural Development in Lacombe (AB, Canada). Grains from three hull-less barley cultivars (waxy, CDC Candle; normal, CDC McGwire; and high-amylose, SH 99250) were obtained from the Crop Development Center at University of Saskatchewan in Saskatoon (SK, Canada). Commercial corn starches of waxy (Amioca), normal (Melojel) and high-amylose (Hylon VII) were obtained from the National Starch Food Innovation in Bridgewater (NJ, USA). The starches used in this study are categorized into three genotype groups; normal (triticale, wheat, corn, and barley), waxy (corn and barley), and high-amylose (corn and barley), depending on the amylose content in their native form. Granular starch hydrolyzing enzyme, Stargen 002 (570 GAU/g) was a gift from Genencor International in Rochester (NY, USA). All other chemicals and reagents used in this study were of ACS grade.

2.2. Grain grinding and starch isolation

Triticale, wheat and barley grains were ground into meals in a Retsch mill (Model ZM 200, Haan, Germany) using a ring sieve with an aperture size of 0.5 mm. Pure starch (purity >95%, w/w) was isolated from the grain meal of triticale, wheat and barley using the procedures reported in earlier publications (Gao, Vasanthan, & Hoover, 2009; Kandil, Li, Vasanthan, Bressler, & Tyler, 2011).

2.3. Molecular characteristics determination of amylopectin (AP) and amylose (AM)

Starch sample (20 mg, dry basis) was solubilized with the addition of 2 mL of 95% (v/v) DMSO followed by heating (85–90 °C) in a water bath for 30 min with vortexing every 5 min. The solubilized starch solution was cooled to room temperature followed by the addition of absolute ethanol (6 mL). The solution was then kept at 4 °C for 2 h, centrifuged (6000 × *g* for 10 min) and the pellet washed with cold ethanol (5 mL). The pellet was then re-solubilized by the addition of 2 M KOH (2 mL) followed by mechanical mixing for 1 h in an ice-bath (tubes containing the samples were covered with ice in a styrofoam box) and then for 15 h at room temperature (~22 °C). The alkaline solution containing starch molecules was diluted with 0.2 M $NaNO_3$ (15 mL) solution, neutralized by 2 M HCL (pH was adjusted between 6.7 and 6.9) and then made up to volume (20 mL) with 0.2 M $NaNO_3$ (starch polymer concentration was 1 mg/mL) followed by filtration through a nylon membrane filter (1 μm) device (Puradisc 25 NYL, Whatman Inc., NJ, USA). An aliquot (50 μL) of the filtrate was injected into an HPSEC-MALLS-RI system. In order to avoid aggregation of dispersed starch molecules, the solubilized starch in KOH solution was neutralized by HCl instantly before each injection.

The HPSEC-MALLS-RI system consisted of an Agilent 1200 HPLC system (Agilent Technologies in Santa Clara, CA, USA) coupled with a multi-angle laser light scattering detector which had a laser wavelength of 658 nm (MALLS, DAWN-HELEOS II, Wyatt Technology in Santa Barbara, CA, USA), and a refractive index detector (RID, Agilent Technologies in Santa Clara, CA, USA). A guard column (Ultrahydrogel™, 6 × 40 mm, Waters Corporation in Milford, MA, USA) and an SEC column (Ultrahydrogel™ Linear, 7.8 × 300 mm, Waters Corporation in Milford, MA, USA) were connected to the HPLC system. The mobile phase used in HPSEC system was aqueous $NaNO_3$ (0.2 M) solution with a flow rate of 0.5 mL/min. The column and RI detector temperatures were maintained at 40 °C and 35 °C, respectively. Before injection of starch samples, two types of dextran with different molecular weight (M_w) were analyzed to test the chromatography system. The M_w of tested dextrans were (2.45×10^5 and 1.01×10^7 g/mol) in agreement with reported values by the manufacturer (Sigma–Aldrich Canada Ltd. in Oakville, ON, Canada). Two injections were completed for each starch type.

The ASTRA software (Version 5.3.4.20, Wyatt Technology in Santa Barbara, CA, USA) was used to collect and analyze data from the HPSEC-MALLS-RI system. A d*n*/d*c* value of 0.146 mL/g for starch was applied in calculations using the Berry extrapolation model with a first-degree polynomial fit (Chen & Bergman, 2007; Rolland-Sabate, Colonna, Mendez-Montealvo, & Planchot, 2007; Yoo & Jane, 2002). The M_w (weight-average molecular weight, g/mol) and R_z (z-average radius of gyration, nm) of AM and AP were automatically calculated by ASTRA, and the average dispersed-molecular density ($\rho = M_w/R_z^3$ in g/mol/nm^3) of AP and AM was calculated according to the method of Yoo and Jane (2002). The branching parameters such, average number of branch points (*B*) (Rolland-Sabate et al., 2007), weight-average degree of polymerization [$DP_w = M_w/162$ (Chen & Bergman, 2007)], average unit chain length [$CL = DP_w/B$ (Rolland-Sabate et al., 2007)], and average degree of branching [$DB = (B/$