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Chemical structures in pyrodextrin determined by nuclear magnetic resonance spectroscopy

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ABSTRACT

Glycosidic linkages in a pyrodextrin were identified by NMR spectroscopy for the first time. Pyrodextrin was prepared by slurrying waxy maize starch at pH 3, filtering and drying at 40 °C to 10–15% moisture content, then heating at 170 °C for 4 h. ^{1}H and ^{13}C NMR resonances of the pyrodextrin were assigned with the assistance of 2D techniques including COSY, TOCSY, HSQC, and HMBC, all measured on a 500 MHz instrument. During dextrinization, native waxy maize starch was hydrolyzed and extensively branched with new glycosidic linkages. The resulting pyrodextrin became 100% soluble in water and produced lower viscosity solutions at 30% solids. There were only 1.2% reducing ends (α-form) detected in the pyrodextrin, but 1,6-anhydro-β-D-glucopyranosyl units accounted for 5.2% of repeating units and they were thought to be at the potential reducing end. New glycosyl linkages including α-1,6, β-1,6, α-1,2, and β-1,2 were identified. The total non-α-1,4 linkages in the pyrodextrin were about 17.8% compared to 5.8% in a maltodextrin prepared by α-amylase digestion. Transglycosidation and depolymerization occurred during dextrinization, and the resulting pyrodextrin was highly branched.

1. Introduction

Pyrodextrin is prepared by thermal treatment of dry starch in the granular form, either with or without acid (Bai, Cai, Doutch, Gilbert, & Shi, 2014; Wurzburg, 2006). Pyrodextrins have been applied in industry as binders, coatings, adhesives (Bhatt, Kumar, & Soni, 2000), oral delivery vehicles (Alvani, Qi, & Tester, 2011) and in food as dietary fiber (Hashizume & Okuma, 2009; Lefranc-Millot, Wils, Roturier, Le Bihan, & Saniez-Degrave, 2009; Maningat & Seib, 2013; Ohkuma & Wakabayashi, 2001; Wurzburg, 2006). Depending on the preparation conditions, pyrodextrin is classified in three categories: white dextrin, yellow or canary dextrin, and British gum (Tomasik, Wiejak, & Palasinski, 1989; Wurzburg, 1986). The physical properties of pyrodextrin are often characterized by color, solubility, alkali-lability, reducing sugar content, viscosity, and β-amylolysis, among others. (Tomasik et al., 1989).

The chemical reactions that occur during dextrinization of starch in air are complex and involve hydrolysis, transglycosidation, repolymerization, and oxidation. Glycosidic linkages of α-1,4 and α-1,6 are partially hydrolyzed during the pre-drying and initial stages of dextrinization (Tomasik et al., 1989). Hydrolysis of starch chains would be expected to be caused by moisture initially in starch as well as water release through dehydration upon heating in presents of hydrochloric acid. Starch hydrolysis results in a decrease in the molar mass, an increase in solubility, and a decrease in viscosity. However, reducing power may increase initially and then decrease during dextrinization (Wang, Kozlowski, & Delgado, 2001). Transglycosidation in starch means less α-1,4 glycosidic linkages with formation of new anomeric linkages (Wurzburg, 1986). A considerable amount of transglycosidation was observed in British gum (Christensen & Smith, 1957; Geerdes, Lewis, & Smith, 1957; Thompson & Wolfrom, 1958) with the formation of α-1,6, β-1,6, α-1,2, and β-1,2 linkages. Methylation studies suggested the formation of new bonds and a branched structure for pyrodextrin (Brimhall, 1944; Christensen & Smith, 1957; Geerdes et al., 1957; Nunes et al., 2016; Siljeström, Björck, & Westerlund, 1989); however, the details of the new glycosidic bonds were not reported. The branched structure of pyrodextrin was suggested to be responsible for an increase in its α-amylase resistance (Laurentin, Cardenas, Ruales, Perez, & Tovar, 2003; Lehmann & Robin, 2007). Repolymerization also was reported in pyrodextrin (Nunes et al., 2016; Wurzburg, 1986; Terpstra, Woortman, & Hopman, 2010). In addition, the formation of 1,6-anhydro-β-D-glucopyranosyl or levoglucosanyl-type, end groups was reported in pyrodextrin (Kroh, Jalyschko, & Haseler, 1996; Lowary & Richards, 1991; Siljeström et al., 1989; Thompson & Wolfrom, 1958; Wolfrom, Thompson, & Ward, 1959). The anhydro end group may act as an intermediate in forming new glycosidic

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linkages (Wolfrom et al., 1959). However the formation of an anhydro end group was opposed by Brimhall (1944), and a methylation study also failed to support that concept (Geerdes et al., 1957).

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool to investigate the structure of carbohydrates including identification and quantification of glycosidic linkages, and determination of α- and β-anomeric configurations (Cui, 2005). It has been successfully applied to characterize the glycosidic bonds of D-glucopyranosyl repeat units by analyzing ^{1}H NMR and ^{13}C NMR spectra (Roslund, Tähtinen, Niemitz, & Sjöholm, 2008; Usui et al., 1973). A ^{13}C NMR spectrum of yellow potato dextrin was reported (McIntyre, Ho, & Vogel, 1990) but not fully analyzed. Levoglucosan, cellobiose and gentiobiose were identified by ^{1}H NMR in heat treated wheat starch (Siljeström et al., 1989). No detailed study on the structure of pyrodextrin by NMR has been reported. Previously, we examined the structural changes of waxy maize starch granules during dextrinization by small-angle and wide-angle X-ray scattering, differential scanning calorimetry, and gel permeation chromatography (Bai et al., 2014). Herein, we report the first detailed NMR study with an interpretation of 1D and 2D-NMR spectra of a pyrodextrin and chemical structural changes that occur during dextrinization of starch. The information gained would be useful in relating the structure of a pyrodextrin to its nutritional and functional properties.

2. Materials and methods

2.1. Materials

Waxy maize starch (Amoica TF) was obtained from National Starch LLC, now Ingredion Inc. (Bridgewater, NJ). α-Amylase (Termamyl, 120L) was obtained from Novozymes (Franklinton, NC), and enzyme activity was 120KNU-T/g. One KNU is defined as the amount of enzyme that converts 5.26 g of starch (Merck Amylum soluble) per hour to an equivalent amount of D-glucose under standard conditions (37.0 °C, 0.0003MCa.$^{2+}$, and pH 5.6). Other chemicals were analytical grade.

2.2. Preparation of pyrodextrin

Waxy maize starch (100 g dry weight) was suspended in water (150 mL), and the slurry was adjusted to pH 3.0 using 0.5 M HCl. The slurry was filtered to recover a starch cake with approximately 50% moisture content. The starch cake was broken into small lumps that were held in an oven at 40 °C for 24 h to a moisture content of 10–15%. The dried starch was ground, passed through a 100-mesh screen, and placed in an open dish. The dish was heated in an oven at 170 °C for 4 h, and the pyrodextrin was kept under ambient conditions overnight, which gave a final moisture content of about 7%. The color of the pyrodextrin was yellow which puts it in the canary class.

2.3. Preparation of maltodextrin

Maltodextrin was prepared from native waxy maize starch as reported by Lumdubwong and Seib (2001) with some modifications. Briefly, α-amylase (1 mL, 120KNU) was diluted to 10 mL using 5 mM $CaCl_2$ solution. An aliquot (1.65 mL) of the enzyme solution was added to 400 mL of 5 mM $CaCl_2$ solution, and the solution was adjusted to pH 6.0–6.4 using 1 M NaOH. The enzyme solution was transferred to a 3-neck flask filled with a mechanical stirring rod, and heated in a water bath at 94 °C. Starch (150 g, dry weight) was slurried in 600 mL of 5 mM $CaCl_2$ solution, and the slurry was adjusted to pH 6.0–6.4 using 0.1 M NaOH. The starch slurry was slowly added to the enzyme solution in 2 min with vigorous agitation. Hydrolysis was continued for 1 h, after which the reaction was terminated by adding 1.0 M HCl to pH 3.0 and holding at 94 °C for 10 min. The flask was then cooled in an ice-water bath to a temperature below 60 °C, and the solution was adjusted to pH 6.0 using 1 M NaOH. The maltodextrin solution was filtered through filter paper, and then freeze-dried. The yield of solids, which was calculated to contain 0.6 g of salts, was 150 g.

2.4. NMR spectroscopy

For NMR analysis, the OH-protons of the pyrodextrins and maltodextrin were exchanged with D_2O (1 mL) twice, followed each time by freeze-drying. The deuterium-exchanged pyrodextrins were dissolved in D_2O at 10 wt% and analyzed by NMR as previously described (Bai and Shi, 2011; Bai, Shi, Herrera, & Prakash, 2011).

The NMR spectra were recorded on a Varian 500 NMR System (Palo Alto, CA) at 25 or 35 °C operating at 499.839 and 125.697 MHz for ^{1}H and ^{13}C, respectively. The NMR spectrometer was equipped with a cryogenic 5-mm probe and a carbon-enhanced triple-resonance inverse detector with pulse field gradient probe. The ^{1}H spectra were collected in 32 individual scans with a sweep width of 16 ppm and a delay time of 1 s. The broadband proton-decoupled ^{13}C spectrum was obtained by the accumulation of 500 scans. ^{1}H-^{1}H 2D homonuclear correlation spectroscopy (COSY) was conducted with 256 transients and 4 scans per transient. Total correlation spectroscopy (TOCSY) was performed with 256 transients and 16 scans per transient. A heteronuclear multiple bond correlation (HMBC) ^{1}H-^{13}C 2D experiment was conducted with 256 transients and 16 scans per transient. A heteronuclear single quantum coherence (HSQC) ^{1}H-^{13}C 2D experiment was conducted with 256 transients and 16 scans per transient in the phase-cycling detection mode. The COSY and HSQC pulse sequences used are part of the "Bio-pack" provided by Varian. Tetramethylsilane (TMS) was used as an internal reference at 0 ppm, and chemical shifts (δ-values) were reported in parts per million (ppm) from the reference.

The level of the α-anomer at the reducing end of a glucan polymer was determined by the equation $\frac{I_{5.20}}{I_{\text{anomeric protons}}} \times 100\%$. Degree of total non α-1,4 linkages branching in the pyrodextrin was calculated as $\frac{I_{5.09}+I_{4.94}+I_{4.4-4.7}}{I_{\text{anomeric protons}}} \times 100\%$.

2.5. Viscosity and solubility

The viscosity of the pyrodextrin was determined by a Brookfield viscometer (RVDVII + Pro, Brookfield Engineering Laboratories, Inc., Middleboro, MA) fitted with a CS4-18 spindle and a SC4-13 RPY chamber. Pyrodextrin solutions of 30% solid content were analyzed at a spindle speed of 100 RPM at 25 °C. Solubility of pyrodextrin was measured as previously described (Bai et al., 2014).

3. Results and discussion

3.1. New bond formation during dextrinization

The ^{1}H NMR spectra of the maltodextrin and the pyrodextrin are shown in Fig. 1. Anomeric protons were well separated and resolved in the low-field region of the spectra between 4.4 and 5.5 ppm. All the other methine and methylene protons were overlapping in the crowded field between 3.5 and 4.0 ppm. New peaks at 5.44, 5.09, 4.75, 4.51–4.60, 4.4–4.5 and 4.1–4.2 ppm were observed in the ^{1}H NMR spectrum of pyrodextrin as compared to that of the maltodextrin. These new peaks resulted from new bonds or linkages formed during dextrinization. One new peak at 4.75 ppm overlapped with the water peak when the spectra were recorded at 25 °C (Fig. 1B and C), but the peak was clearly resolved when the spectrum was recorded at 35 °C (Fig. 1D).