Carbohydrate Polymers 82 (2010) 376–383

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Amylose involvement in the amylopectin clusters of potato starch granules

Debora Saibene [a], Koushik Seetharaman [b,*]

[a] Department of Food Science, Pennsylvania State University, University Park, PA 16802, United States
[b] Department of Food Science, University of Guelph, Canada

ARTICLE INFO

Article history:
Received 8 February 2010
Received in revised form 14 April 2010
Accepted 26 April 2010
Available online 4 May 2010

Keywords:
Amylose
Lintners
Iodine
Starch granules

ABSTRACT

The granular organization of common corn and potato starch is characteristically different. The objective of this study was to investigate iodine complex formation with starch lintners from common corn starch (CCS) and potato starch (PS) litners as a function of water content. Starches were subjected to mild acid hydrolysis (lintnerization). Size exclusion chromatography of the lintners indicated that the linear chains remaining after lintnerization had smaller degree of polymerization in PS lintners than in the corresponding CCS lintners. For both CCS and PS, the absorbance intensity and the wavelength of maximum absorption (λ_{max}) of molecularly dispersed starches, in diluted iodine solution, decreased with increasing lintnerization extent. When the granular lintners were exposed to iodine vapor, following equilibration to different moisture contents, the reduction in the iodine binding was evident in PS lintners but not in CCS lintners. Furthermore, the iodination partially destroyed the crystallinity of native PS granules but not that of CCS granules. However, B-type crystallinity was still evident in PS lintners. This behavior was attributed to different location of amylose within starch granules, supporting the involvement of amylose in the B-type crystallites of PS, and the independence of amylose from the A-type crystallites of CCS.

Published by Elsevier Ltd.

1. Introduction

When observed by optical microscopy, starch granules consist of concentric rings of alternating semi-crystalline and amorphous regions, referred to as growth rings (French, 1984; Jenkins & Donald, 1995). The semi-crystalline growth rings are comprised of alternating amorphous and crystalline 'lamellae'; each lamella is approximately 9–10 nm thick (Jenkins, Cameron, & Donald, 1993). In the crystalline lamellae, the external chains of amylopectin are associated in double helices and are packed together in an array to form clusters (Gallant, Bouchet, & Baldwin, 1997). The amylopectin clusters are considered responsible for the crystalline structure (A- and B-type) of starch. The branch points of the amylopectin molecules are thought to reside in the amorphous lamellae.

Starch granules are hydrolyzed in dilute acids. Acid hydrolysis can be performed with hydrochloric acid, producing lintnerized starches (Lintner, 1886). The amorphous regions (amorphous growth rings and amorphous lamellae) of granular starch are less dense and more susceptible to chemical and enzymatic modification than the crystalline or semi-crystalline regions (Biliaderis, 1998). Diffusion of small water-soluble molecules in the granule also are more likely to occur through the amorphous regions (French, 1984). Therefore, when the starch granule is treated with acid, the amorphous regions of the granule are preferentially hydrolyzed, leaving intact the more resistant crystalline regions of the granule (Gallant et al., 1997).

The structure of the starch granule depends on the way in which amylose and amylopectin are associated and distributed throughout the starch granule. In contrast to the defined location and role of amylopectin, the location and role of amylose within the granule is poorly defined. An original study (Jane & Shen, 1993), based on cold gelatinization in a calcium chloride solution, proposed that amylose is more concentrated in the periphery of potato starch granule. In contrast, Tatge, Marshall, Martin, Edwards, and Smith (1999), based on the investigation of amylose synthesis in transgenic potato starch granules, suggested that amylose is largely confined to a central region of the granule. Jenkins and Donald (1995) applied small-angle X-ray scattering techniques to investigate the effect of varying amylose content on the internal structure of maize, barley and pea starch species. They hypothesized that amylose is predominatly located in the amorphous growth rings, and that the interaction between amylose and amylopectin in these amorphous regions may be the cause of decreased crystallinity. The degree of interaction between amylose and amylopectin may depend on the botanical source of the starch (Oates, 1997), with amylose and amylopectin more closely associated in potato starch than in corn starch (Hoover & Vasanthan, 1994; Saibene, Zobel, Thompson, & Seetharaman, 2008; Zobel, 1988). Gerard, Colonna, Buleon, and Planchot (2002), used mild acid hydrolysis to investigate the location of amylose with respect to the amorphous and/or crystalline regions in maize starch mutants; and reported a greater

* Corresponding author at: Department of Food Science, Guelph, ON N1G 2W1, Canada. Tel.: +1 519 824 4120; fax: +1 519 824 6631.
E-mail address: kseethar@uoguelph.ca (K. Seetharaman).

0144-8617/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2010.04.070

interaction of amylose and amylopectin in high-amylose maize starches than in common corn starch. However, as pointed out by Jane (2006), the present knowledge concerning the location of amylose relative to the amylopectin molecules in common corn starch is contradictory. Based on cross-linking studies, Jane, Xu, Radosavljevic, and Seib (1992) and Kasemsuwan and Jane (1994) suggested the interaction of amylose and amylopectin in common corn starch. On the other hand, Zobel (1988) suggested that amylose exists separate from amylopectin in common corn starch based on criteria of amylose extractability, ease of amylose complex formation, susceptibility of amylose to enzyme attack, and gel setback. Clearly, the location of amylose within starch granules remains one of the unknown facts required to complete our picture of the internal structure of the starch granule.

The ability of starch to complex with iodine is commonly used for the characterization of starch molecules from various botanical sources (Knutson, 1999). The amylose–iodine complex consists of a single helical inclusion complex, which can crystallize in a V-type crystalline pattern (Rundle & French, 1943). The traditional spectrophotometric approach consists of measuring absorbance as a function of wavelength for dispersed starches in a diluted iodine solution. The color and wavelength of maximum absorbance (λ_{max}) of the complex vary accordingly to the degree of polymerization (DP) of the polymer chain. As the length of the glucan chain increases, the λ_{max} also increases (Banks & Greenwood, 1975). For granular starches, light scattering as well as absorption occurs. In two previous publications (Saibene & Seetharaman, 2006; Saibene et al., 2008) we investigated iodine complex formation with starch molecules in native granular starches as a function of water content by using the Kubelka and Munk theory (Kubelka & Munk, 1931). According to this theory, a powder sample such as granular starch is considered as a continuous, turbid medium. When the incident light strikes on the surface of a sufficiently thick layer of starch granules, a fraction of light that is scattered is described by a scattering coefficient (S), while the fraction of light that is absorbed is described by an absorption coefficient (K). The ratio K/S is determined by measuring the reflectance (R) of the sample (Kubelka & Munk, 1931; Lindberg & Laude, 1974).

The objective of this study was to investigate iodine complex formation with starch molecules in common corn starch (CCS) and potato starch (PS) litners as a function of water content. The goal of this study was to potentially highlight structural differences in the granular arrangement of CCS and PS.

2. Materials and methods

2.1. Materials

Commercial starch from common maize endosperm (Melojel®) was a gift from National Starch and Chemical Company (Bridgewater, NJ). Commercial starch from potato (Kosher® FGPS) was a gift from Avebe (Veendam, The Netherlands). These starches are referred to as CCS and PS, respectively.

Drierite® (W.A. Hammond Drierite Company Ltd., Xenia, OH), $MgCl_2$ (EMD Chemicals Inc., Gibbstown, NY), NaCl (Fisher Chemicals Inc., Fair Lawn, NJ), K_2SO_4 (EDM Chemicals Inc., Gibbstown, NY), and sodium azide (EM Science, Gibbstown, NY) were purchased. Iodine crystals were purchased from J.T. Baker (Phillipsburg, NJ).

2.2. Starch lintnerization

Starch granules (CCS and PS) were treated with 2.2 M HCl (5 g starch/100 mL) at 29 °C. The slurry was stirred periodically everyday.

Small aliquots (2 mL), taken at intervals, were centrifuged at 1000 × g for 5 min, and the supernatants analyzed for carbohydrates by using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Following treatment for 2 h, 2 days, 6 days, and 21 days a sample of the slurry was centrifuged (15 min at 1000 × g), and the granular residues (lintners) were washed with water (2×), neutralized with 0.1 N NaOH, washed with water (3×), and finally washed once with acetone. The lintners were then dried at room temperature, ground in a mortar, and sieved (openings 125 μm).

2.3. Sepharose CL-2B chromatography

Native starches and lintners (1 g db) were dispersed in 20 mL of 90% (v/v) DMSO at room temperature with constant stirring overnight. Following dispersion, 4 vol of ethanol were added and the mixture was centrifuged at 1000 × g for 15 min at room temperature. The supernatants were discarded and the pellets were washed once with ethanol and once with acetone. The precipitates were then dried at room temperature.

Dispersed starch samples (15 mg) were dispersed in 2 mL of 0.1 M NaOH for 1–2 days. The dispersed starches were then diluted with 5 mL of water and loaded into a Sepharose CL-2B (Supelco Chromatography Products, Bellefonte, PA) SEC column (74 cm × 2.5 cm, Pharmacia Fine Chemicals, Sweden) using gravity flow. The nominal fractionation range for dextrans is 100,000–20,000,000 MW.

For each sample, 550 mL of eluent was collected as 5 mL fractions, at a flow rate of 20–30 mL/h. The void volume and salt volume of the column were determined using a mixture of 1 mg of waxy corn starch and 1 mg of glucose. Every third SEC fraction and every SEC fraction within 10 fractions of the peak were examined for total carbohydrate and iodine binding λ_{max}, according to Klucinec and Thompson (1998).

2.4. Iodine binding of dispersed starches

The iodine binding of the dispersed starches was determined by using the method described by Klucinec and Thompson (1998). All samples were scanned from 400 to 800 nm by using a Heλios Alpha UV–visible Spectrophotometer (Thermo Spectronic, Rochester, USA). The blue value of the starches was defined as the absorbance at 635 nm. The λ_{max} was the peak absorbance value over the range of wavelengths examined.

2.5. K/S spectra of granular samples exposed to iodine vapor

2 g of starch or lintner samples were equilibrated to the respective water activity (a_w), with final values lower than 0.15, and to 0.33, 0.75, and 0.97 a_w using Drierite®, or saturated solutions of $MgCl_2$, NaCl, and K_2SO_4 (Greenspan, 1977), as described by Saibene and Seetharaman (2006). Following equilibration, the moisture content of the samples was measured according to the AACC method 44-15A (AACC, 2000). To determine the iodine binding, a thin layer of the equilibrated starch sample (0.2 g) was spread in a standard plastic weighing dish, placed in the corresponding a_w desiccator, and exposed to iodine vapor generated from 2 g of iodine crystals for 24 h at room temperature. The K/S values over a wavelength range from 400 to 700 nm of the samples after exposure to iodine vapor were measured as described in Saibene and Seetharaman (2006) with a CM 3500-d Spectrophotometer (Konika Minolta, Mahwah, NJ).

2.6. X-ray powder diffraction

A Kα 1 = 4.54056 Å and Kα 2 = 1.54439 Å radiation was produced by the copper X-ray tube of a Scintag Pad V X-ray powder diffrac-