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ScienceDirect
Carbohydrate Polymers 73 (2008) 532–540
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Effects of protein on crosslinking of normal maize, waxy maize, and potato starches

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Received 6 March 2007; received in revised form 13 November 2007; accepted 21 December 2007
Available online 15 January 2008

Abstract

Channels of maize starch granules are lined with proteins and phospholipids. Therefore, when they are treated with reagents that react at or near the surfaces of channels, three types of crosslinks could be produced: protein–protein, protein–starch, starch–starch. To determine which of these may be occurring and the effect(s) of channel proteins (and their removal) on crosslinking, normal and waxy maize starches were treated with a proteinase (thermolysin, which is known to remove protein from channels) before and after crosslinking, and the properties of the products were compared to those of a control (crosslinking without proteinase treatment). After establishing that treatment of starch with thermolysin alone had no effect on the RVA trace, three reaction sequences were used: crosslinking alone (CL), proteinase treatment before crosslinking (Enz-CL), proteinase treatment after crosslinking (CL-Enz). Two crosslinking reagents were used: phosphoryl chloride ($POCl_3$), which is known to react at or near channel surfaces; STMP, which is believed to react throughout the granule matrix. Three concentrations of $POCl_3$ (based on the weight of starch) were used. For both normal maize starch (NMS) and waxy maize starch (WMS) reacted with $POCl_3$, the trends were generally the same, with apparent relative degrees of crosslinking indicated to be CL-Enz = CL > Enz-CL, but the effects were greater with NMS and there were differences when different concentrations of reagent were used. The basic trends were the same when potato starch was used in the same experiments. Crosslinking with STMP was done both in the presence and the absence of sodium sulfate (SS). Both with and without SS and with both NMS and WMS, the order of indicated crosslinking was generally the same as found after reaction with $POCl_3$, with the indicated swelling inhibition being greater when SS was present in the reaction mixture. Examination of the maize starches with a protein stain indicated that channel protein was removed by treatment with thermolysin when the proteinase treatment occurred before crosslinking with either $POCl_3$ or STMP, but only incompletely or not at all if the treatment with the proteinase occurred after crosslinking. Because the crosslinking reactions were less effective when the protein was removed, the results are tentatively interpreted as indicating that they involved protein molecules, although there may not be a direct relationship.

Keywords: Crosslinked starch; Normal corn starch; Potato starch; Waxy corn starch; Starch granule protein

1. Introduction

Previously, starch protein was categorized as surface and granule-bound (Gillian, Sargeant, & Schofield, 1981). In isolated maize starch, the external surface protein is zein, which can be removed by treatment with thermolysin) (Han, Benmoussa, Gray, BeMiller, & Hamaker, 2005; Tester, Yousuf, Kettlitz, & Röper, 2007). Among granule-bound proteins are the waxy protein (granule-bound starch synthase, GBSS) (Nakamura, Yamamori, Hirano, & Hidaka, 1993), starch synthase (SSI), and starch branching enzyme IIb (SBEIIb) (Mu-Forster et al., 1996). They are extracted only when the starch granules are considerably swollen (Sano, 1984). Like the external surface protein, the protein components of the lining of granule channels of normal and waxy maize starch (Fannon, Gray, Guna-

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0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.12.022

wan, Huber, & BeMiller, 2003, 2004; Han, Gray, Huber, & BeMiller, 2006) can be removed by treatment with thermolysin (Han et al., 2005).

A widely used type of chemical derivatization used to make modified food starches is that which introduces distarch phosphate crosslinking. Phosphoryl chloride ($POC1_3$) is the most widely used reagent for crosslinking. Its reaction with starch is very rapid, resulting in reactions at or near the external and channel surfaces of corn starch granules (Gray & BeMiller, 2004; Huber & BeMiller, 2001). Because it reacts at or near the surfaces of channels, it could produce three possible types of crosslinks: protein–protein, protein–starch, or starch–starch. Reactions with sodium trimetaphosphate (STMP) and epichlorohydrin (EPI), two additional crosslinking reagents, are much slower and are, therefore, believed to occur more evenly throughout granules. Hirsch and Kokini (2002) compared reactions of waxy maize starch with $POC1_3$, STMP, and EPI and reported that $POC1_3$-generated crosslinks appeared to be much more effective in preventing granule swelling than were those resulting from reaction with STMP or EPI.

Reagents contact granule surfaces, including channel surfaces, before penetrating into the matrix; so it was hypothesized that removing the protein lining granule channels of maize starch could affect chemical modification. The objective of this research was to determine the effect of channel and surface proteins on modification of normal and waxy maize starches. For crosslinking, phosphoryl chloride ($POCl_3$) and STMP were used. Pasting characteristics were used to determine the degree of reaction. Potato starch, which is devoid of surface pores (Fannon, Hauber, & BeMiller, 1992) and channels, was reacted in the same ways for comparison.

2. Materials and methods

2.1. Materials

Commercial normal and waxy maize (Tate & Lyle North America, Decatur, IL) and potato starches (Penford Food Ingredients, Englewood, CO) were used. A CBQA protein quantitation kit containing 3-(4-carboxybenzoyl)quinoline-2-carboxyaldehyde was purchased from Molecular Probes (Eugene, OR). Thermolysin was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO).

2.2. Crosslinking of starch with phosphoryl chloride ($POCl_3$)

Starch (20 g, db) was reacted with three levels of $POCl_3$ (0.05%, 0.075%, and 0.1% [0.53, 0.79, and 1.06 mmol/glucosyl unit] based on the dry weight of starch) at pH 11.2 for 1 h at 25 °C. Total slurry volume was 40 mL. Reaction pH (11.2) was maintained by addition of 1 M NaOH using a pH-stat autotitrator (Copenhagen, Denmark). After reaction, the starch slurry was neutralized and recovered by vacuum filtration, washed with water and absolute ethanol, and air-dried.

2.3. Crosslinking of starch with sodium trimetaphosphate (STMP)

The method described by Lim and Seib (1993) was used.

2.4. Incubation of starch with thermolysin

Treatment of starch granules with thermolysin (Sigma Chemical Co., St. Louis, MO) was conducted as described by Mu-Forster and Wasserman (1998).

2.5. Confocal laser scanning microscopy (CLSM)

The procedures described by Han et al. (2005) were used.

2.6. RVA analysis

The pasting characteristics of the starches were determined with a Rapid Visco-Analyser (Model 4, Newport Scientific, Warriewood, Australia) using standard profile 1. A 13-min analysis was used: equilibration to 50 °C for 1 min, heating to 95 °C in 222 s, holding at 95 °C for 150 s, cooling to 50 °C in 228 s, and holding at 50 °C for 120 s. Unmodified and treated starches (2.1 g, db) and 27.9 g of distilled water were combined and stirred in the aluminum sample container for 20–30 s before inserting the container into the instrument. Analyses were done in triplicate.

3. Results and discussion

Starch samples treated with thermolysin (2 mg/g starch) and without treatment (1 g starch only) were analyzed by the Somogyi–Nelson method (Wood, 1994) to check the extent of starch hydrolysis due to contamination of the thermolysin preparation with amylase. The amount of reducing sugar (determined using D-glucose as a reference) was 111 μg/g starch and 22 μg/g starch, respectively, for the starches with and without thermolysin treatment. Although this degree of hydrolysis was thought to be insignificant, to minimize any effect of depolymerization, we determined the approximate minimal concentration of enzyme required (using CLSM); 1 mg/g starch was the amount determined and used thereafter.

3.1. Confocal laser scanning microscopy (CLSM)

Thermolysin is a metaloproteinase that catalyzes hydrolysis of proteins at both protein–membrane and protein–carbohydrate interfaces (Sigma–Aldrich Chemical Co., 1999). Using this enzyme, the main surface protein of starch granules, zein, and most of the channel protein could be removed in a 30-min treatment (Han et al., 2005). Fig. 1 contains CLSM pictures of (1) native and