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Preparation of starch–poly–glutamic acid graft copolymers by microwave irradiation and the characterization of their properties☆

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ABSTRACT

Graft copolymers of waxy maize starch and poly-γ-glutamic acid (PGA) were produced in an aqueous solution using microwave irradiation. The microwave reaction conditions were optimized with regard to temperature and pH. The temperature of 180 °C and pH7.0 were the best reaction conditions resulting in a PGA graft of 0.45% based on nitrogen analysis. The average graft content and graft efficiency for the starch–PGA graft copolymer prepared at 180 °C and pH7.0 were 4.20% and 2.73%, respectively. The starch–PGA graft copolymer produced at 180 °C and pH7.0 could absorb more than 20 times its own weight amount of water and form a gel. The preliminary rheology study revealed that the starch–PGA graft copolymer gel exhibited viscoelastic solid behavior while the control sample of waxy starch showed viscoelastic liquid behavior.

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1. Introduction

Starch is a low cost, multipurpose, biodegradable biopolymer. Depending on the reagent and parameters used, modifying starch by grafting a monomer onto it can create a graft copolymer with increased water absorbent capabilities, thermal stability, and increased viscosity. Starch graft copolymers can be applied in many ways including waste water treatment, in cosmetics, as flocculants (Pal, Sen, Ghosh, & Singh, 2012), and as super absorbers; and therefore greatly attract scientists' interests (Jyothi, 2010). Various modified starch-based copolymer preparations have been reported and described (Fanta, 1996). One of the processing methods to prepare starch graft copolymers is microwave irradiation or heating. Microwave assisted synthesis is a useful tool for a wide range of chemical reactions. This is due, in part, to its precisely controlled temperature and pressure environment. Processes performed under microwave heating offer several advantages over conventional heating, such as reduced reaction times, larger yields, more eco-friendly, and less auxiliary reactions. This creates an end product that is more pure and not contaminated by other reagents (Hoogenboom & Schubert, 2007; Petit, Reynaud, & Desbrieres 2015). Microwave heating has been used more and more in polymer synthesis during recent years (Kempe, Remzi Becer, & Schubert, 2011; Singh, Kumar, & Sanghi, 2012). Zhou, Song, and Parker (2006) reported a method by which starch-based foams were prepared by microwave heating. Other starch derivatives have also been prepared utilizing microwaves (Shogren & Biswas, 2006). Additional methods of producing starch-based graft copolymers using microwave heating have been reported (Singh, Tiwari, Pandey, & Singh, 2007; Mishra, Mukul, Sen, & Jha, 2011; Singh, Tiwari, Pandey, & Singh, 2006; Kumar, Setia, & Mahadevan, 2012; Chang et al., 2009). However, the method used in this study is a narrower, more refined procedure for graft copolymer polymerization. Poly-γ-glutamic acid (PGA) is a water soluble poly amino acid. It is not only a biodegradable, but also an edible biopolymer. The unique properties of the PGA allow it to have many potential applications in drug delivery, food, water treatment, cosmetics and other fields (Bajaj & Singhal, 2011).

The purpose of this work is to develop a starch-based biopolymer that is biodegradable, edible, and absorbs plenty of water while still retaining its gel properties; this new starch-based biomaterial could have a variety of useful applications. For example, starch grafted PGA could be used as stable hydrogel particles for controlled or targeted release of lipophilic and hydrophilic bioactive

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agents in the GI tract (Zhang, Zhang, Chen, Tong, & McClements, 2015). Waxy starch, which is composed of highly branched amylopectin, was used in this study rather than normal, less expensive starch because the amylopectin forms a more stable paste or gel-like material (Doublier & Llamas, 1993; Zhang et al., 2015). PGA was adopted for this study because it is water soluble; and thus it should be miscible with starch. Microwave irradiation has been used to produce starch–PGA copolymers (Pal et al., 2012); however, the reaction first grafted glutamic acid onto amylose chains and then polymerized the monomer. This new procedure uses microwaves to graft PGA onto amylopectin.

Properties including water absorption, water solubility, graft content, graft efficiency, and rheological behavior are reported.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all materials were obtained from commercial sources and used as supplied. Waxy maize corn starch (7350) was obtained from Tate & Lyle (Decatur, IL, USA). The ambient moisture content of the starch was 11.2% by weight, using gravimetric measurements (AACC 44.01.01). Acetanilide Calibration Standard was purchased from Perkin Elmer (PN 0240-1121–C 71.09% H 6.71% N 10.36%). Enzymes (Alpha-amylase and amyloglucosidase) were obtained from Megazyme (Wicklow, Ireland).

2.2. Poly-γ-glutamic acid (PGA) preparation

2.2.1. Strain information and preservation

Bacterial cultures were cultivated to produce PGA. *Bacillus licheniformis* ATCC 9945a was purchased as a lyophilized powder from the American Type Culture Collection (Manassas VA) and was reconstituted in sterile ATCC Medium 21 (Bacillus Medium) according to ATCC instructions (http://www.atcc.org/Products/All/9945a.aspx#generalinformation). The medium contained the following in g/L: K_2HPO_4, 0.5; Ferric Ammonium Citrate, 0.5; $MgSO_4$, 0.5; Glycerol, 20.0; Citric Acid, 2.0; L-Glutamic Acid, 4.0 (all chemicals were at least reagent grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA). The pH of the medium was adjusted to 7.4 ± 0.2. Mucoid colonies were aseptically selected from Bacillus Medium plates solidified with 1.5% agar and streaked onto fresh Bacillus plates and stored at 4 °C for use as inocula for the PGA production. All plates were re-streaked on a monthly basis to maintain mucoid phenotype. In addition, *B. licheniformis* ATCC 9945a was maintained in a cryogenic state in Bacillus Medium containing 10% (v/v) glycerol at −80 °C.

2.2.2. PGA production

PGA production was achieved at the bench-top scale in a 10-L volume using a New Brunswick Scientific BioFlo 3000 (now part of Eppendorf Inc., Enfield, CT). The medium (Medium E) used for PGA synthesis was previously described by Birrer, Cromwick, and Gross (1994). The inoculum was prepared by aseptically transferring a single mucoid colony from the refrigerated stock plates (described in the previous section) into 10 ml of Media E (pH 7.4) contained in a sterile test tube and incubated at 37 °C, 250 rpm for 24 h in a shaker incubator. The entire 10 ml sample was then aseptically transferred into 50 ml of Media E and incubated again as described above. After an additional 24-h the 50 ml culture was aseptically transferred to 1 l of Media E and the culture then allowed to grow under identical conditions noted above for another 24 h after which the entire 1 l inoculum was aseptically centrifuged and the supernatant discarded. The pelleted cells were resuspended in 150 ml of fresh Media E from the fermenter itself and then aseptically poured back into the 10-l fermenter as inoculum for PGA production. The fermentation conditions were set at 37 °C. The media was sparged with sterile air at 1 l/min and stirred with an impeller operating at 600 rpm. The pH of the media, at the start of culturing, was 7.4. The fermentations were conducted for 96 h after which the entire culture was centrifuged (10,000 × *g*, 4 °C, 20 min) to separate the cells from the PGA-containing supernatant. The cell pellets were washed twice with deionized water to achieve maximum PGA recovery. The water washings were added to the supernatant and the entire aqueous phase (supernatant) was lyophilized to dryness. The residual PGA polymers were resuspended in one-quarter volume deionized water and then precipitated into excess cold ethanol. The PGA was then recovered and dried under vacuum to a constant weight. In order to confirm that the PGA was pure, amino acid analyses were done as described in the method below.

2.3. Amino acid test to confirm the purity of PGA

Dehydrated samples were hydrolyzed in 6N HCl containing a small amount of phenol. The hydrolysis flasks were extensively purged of oxygen using a PicoTag workstation (Waters Corp., Milford, MA, USA), and then incubated at 110 °C for 20 h. Hydrolyzed samples were filtered, dried under vacuum, and derivatized with AccQFluor reagent (Waters) following the manufacturer's directions. Chromatography was performed using procedures described as 'system 1' by Cohen and De Antonis, with α-aminobutyric acid as an internal standard. Separation was achieved using an AccQTag C18 reverse-phase column (Waters); detection by fluorescence used excitation with 250 nm light and measured emission at 395 nm. Hydrolysis, derivatization, and analysis of each sample were performed in triplicate (Cohen & De Antonis, 1994).

2.4. Preparation of starch–PGA graft copolymer

A 2% (wt.%) suspension of waxy maize starch (dry weight) using nanopure water (Millipore 18.2 MΩ cm) was placed in a pressure vessel and gelatinized by microwave radiation which heated the suspension from 23 °C to 140 °C in 5 min. The gelatinized paste was then freeze dried and stored for further use. Attempting to graft PGA without pregelatinising the waxy starch will not yield measurable grafting of the two materials. A 2% (wt.%) solution of PGA using nanopure water was dissolved and freeze dried. The freeze dried, microwaved starch and freeze dried PGA were the stock material for the preparations.

A 2% solution of the stock freeze-dried waxy maize starch was then combined with a 2% solution of the stock freeze-dried PGA (adjusted to pH 6.0) at a 1:1 ratio. Combined samples were then heated in open vessels for 2 h at 120 °C, 140 °C, 160 °C, 180 °C and 240 °C, respectively. Microwave reactions were carried out using a Initiator Exp Microwave (Biotage Inc., Charlotte, NC), and an Ethos MicroSYNTH 1600 microwave labstation (Milestone Inc., Shelton, CT, USA). Reactions were performed in a 20 ml pressure vessel (354833) in the Biotage microwave oven and in a 270 ml and 500 ml Teflon pressure vessel (45161T and HPR3600, respectively) in the Milestone microwave oven. There were no differences between the products made using either of the microwave systems. The two samples that had the highest water absorption qualities were repeated, this time with polyglutamic acid adjusted to a pH of 4.0, 5.0, 7.0, and 9.0 with 0.5 M NaOH and 0.5 M HCl where needed. The native pH of the sample was approximately 6.5. Optimum temperature was determined by the sample that had the highest water absorption traits. All results are then compared, and the sample with the highest water absorption properties and highest nitrogen content were chosen. Un-reacted or soluble starch and PGA were removed from the sample pellet by washing 2× with nanopure water, centrifuging, and decanting the supernatant, leaving behind the gel-like starch–PGA copolymer. During the washing of