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Short communication

HRP-mediated synthesis of starch–polyacrylamide graft copolymers

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ABSTRACT

Starch was reacted with acrylamide in water in the presence of horseradish peroxidase (HRP) catalyst/H_2O_2/2,4 pentanedione to give starch–polyacrylamide graft copolymers.

1. Introduction

Modified starch-based polymers can be engineered for specific properties by combining starch with synthetic polymers through graft copolymerization (Fanta, 1996; Fanta & Doane, 1986; Fanta & Bagley, 1977; Willett & Finkenstadt, 2005). Polyacrylamide grafted starch has been considered for applications in areas such as superabsorbent (Kiatkamjornwong, Mongkolsawat, & Sonsuk, 2002; Wu, Lin, Zhou, & Wei, 2000) paper-making additives (Heath, Hofreiter, Ernst, Doane, & Hamerstrand, 1975; Lu, Lin, & Cao, 2003; Yeng, Tahir, Chiang, & Yunus, 2004), and textile sizing (Hebeish, El-Rafie, Higazy, & Ramadan, 1996). So far there are over a hundred papers and tens of patents (Luebke, 2000) issued for graft copolymerization. All of these used oxidants such as ceric ammonium nitrate or ammonium persulfate as catalyst. We would like to report a process where we replaced the inorganic oxidants by a natural enzymatic catalyst (HRP). At room temperature and in water HRP/H_2O_2/2,4 pentanedione catalyzed free radical grafting of acrylamide onto starch. Starch–polyacrylamide graft copolymers had graft M.Wt in the range of 100–200 K with good product recovery and a grafting efficiency of 33–65%. HRP is shown to be a viable alternative to conventional inorganic free radical catalysts.

In the past two decades there has been tremendous interest to develop enzymatic polymerizations where enzymes catalyze the building of polymers from monomers (Gross et al., 1998, Gross, Kumar, & Kalra, 2001; Kobayashi, Uyema, & Kimura, 2001). Enzymes are environmentally friendly green catalysts that operates under mild condition, very often in water. HRP enzyme is an oxidoreductase acting on hydrogen peroxide as oxidant and on several reducing substrates such as hydroquinone, catechol and beta diketones (Teixeira, Lalot, Brigodiot, & Marechal, 1999) etc. HRP biocatalytic properties have been widely used to polymerize phenol (Ikeda, Sugihara, Uyama, & Kobayashi, 1996; Ikeda, Uyama, & Kobayashi, 1996), aniline (Bruno et al., 1995) and methyl methacrylate (Gross & Kalra, 2000) monomers.

Derango et al. utilized HRP and other oxidase to catalyze free radical polymerization of vinyl monomers (Derango, Chiang, Dowbenko, & Lasch, 1992). However, the polymers were not well characterized. Free radical polymerization of acrylamide in water initiated by the system HRP/H_2O_2/RH where RH is 2,4-pentanedione was reported by Lalot (Emery, Lalot, Brigodiot, & Marechal, 1997; Teixeira et al., 1999). Laccase catalyzed acrylamide polymerization was reported by Kobayashi (Ikeda, Tanaka, Uyama, & Kobayashi, 1998). Gross reported the copolymerization of acrylamide with sodium acrylate (Gross & Kalra, 2002). We would like to report for the first time the simultaneous polymerization and grafting of acrylamide onto starch catalyzed by HRP/H_2O_2/RH.

The polymerization of the acrylamide by HRP has been well known. However the formation of polyacrylamide chain on the starch backbone to form the graft copolymer is novel and has both academic and commercial interest. It is assumed that the grafts are prepared by first generating free radicals on starch and then allowing these free radicals to serve as macro initiators for vinyl or acrylic monomers.

2. Results and discussions

All HRP catalyzed reactions were carried out at a temperature of 30 °C and pH of 7. Thus, the variable parameters for the reactions were the amount of starch, acrylamide and hydrogen peroxide. The success of the reactions were defined by conversions, graft

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content, graft efficiency and M.Wt. Conversion implies the percentage of acrylamide (AAm) monomer that was converted into polyacrylamide (PAAm). Graft content defines how much PAAm is attached to starch. It is postulated that peroxide activated HRP oxidizes PDO to a free radical which then abstracts a proton from starch backbone to give carbonyl radicals which in turn initiates the grafting reaction and copolymerization. The molecular weight trend follows the expected norms for free radical polymerization where higher monomer concentration or lower initiator concentration led to higher molecular weight. All the data presented in the table are average value of 3–5 independent reactions.

% starch, % AAm, ul of H_2O_2	Conversion (%)	Graft content (%)	Graft efficiency (%)	Wt Average MW ($x10^{-3}$)	Glucose units/ PAAm graft
1. 5, 5, 80 μl	53.3	20.1	57.3	195	2400
2. 5, 5, 400 μl	89.2	23.6	33.3	144	1400
3. 10, 5, 80 μl	20.0	5.9	65.7	308	15000
4. 5, 2.5, 80 μl	48.2	9.7	44.9	99	2800

Conversion, graft content and grafting efficiency were calculated by:
Conversion: $Wt_q \times 100 \times N_q/19.72/$Weight of AAm.
Graft content: $100 \times N_{ext}/19.72^*$.
Graft efficiency: $f \times N_{ext}/N_q$.
Wt_q = product weight after removal of AAm by extraction with ethanol.
N_q = nitrogen content after removal of AAm by extraction with ethanol.
N_{ext} = nitrogen content after removal of homopolymer PAAm by extraction with 30% ethanol.
f = insoluble weight fraction as % $100 \times Wt_{insoluble}/Wt_{soluble} + Wt_{insoluble}$ Grafting efficiency is based on the polymerized monomer, i.e. the ratio of insoluble PAAm to total PAAm.
*Nitrogen content of acrylamide (AAm) is 19.72%.

Starch was removed from the extracted graft copolymer using amylase hydrolysis prior to size exclusion chromatography (SEC) (Willett & Finkenstadt, 2003).

3. Experimental

3.1. Materials

Waxy maize starch (10.4% water content) was used. Peroxidase type 2 from horseradish was purchased from Sigma. All other chemicals were obtained from Aldrich Chemical Co. and used as is.

3.2. Reaction procedure

In a typical experimental procedure, 6.271 g waxy starch in 90 ml water was gelatinized via microwave (Ethos 1600) by heating the mixture up to 140 °C in 5 min and cooling to room temperature. Then 10 ml of 0.5 M potassium phosphate buffer pH 7.0 was added to produce a 0.05 M solution. A portion (26.56 g) of the mixture was then pipetted to125 ml ground glass flask. To the flask also added were 2.5 ml 50% acrylamide solution, and 52 μl of 2,4 pentanedione. Reaction mixture was capped with a rubber septum and placed under a nitrogen purge for 30 min and 750 μl horseradish peroxidase stock (50 mg/ml H_2O) and 80 μl of 0.3% H_2O_2 injected via syringe through the septum while still under nitrogen purge. The reaction mixture was left in incubator shaker for 6 h at 30 °C, 175 rpm and was poured into 200 ml of absolute ethanol to precipitate the product. In addition to quenching the reaction, the ethanol removed unreacted acrylamide (AAm) monomer. The product was filtered and again stirred overnight with 150 ml of absolute ethanol. Product was refiltered and dried in a vacuum oven at 105 °C for overnight. Weight of the recovered solid was termed as Wt_q and nitrogen content was termed as N_q. Approximately 500 mg of the unextracted sample was stirred overnight in 40 ml of 30% ethanol/70% H_2O (vol/vol) to remove polyacrylamide (PAAm) homopolymer. Mixture centrifuged at 3200 rpm for 10 min. Supernatant was transferred to a weighted evaporation dish and dried in 105 °C forced air oven overnight to give soluble sample with weight designated as $Wt_{soluble}$. The tube containing the insoluble portion was placed in 105 °C vacuum oven and dried overnight. This was termed as insoluble sample. Its weight was expressed as $Wt_{insoluble}$ and its nitrogen content was N_{ext}. FTIR spectra of samples after extraction (not shown) display the expected amide I (1675 cm^{-1}) and amide II (1615 cm^{-1}) carbonyl absorption bands from grafted polyacrylamide, consistent with our previous results (Willett & Finkenstadt, 2003). Nitrogen contents were measured using a Perkin Elmer 2400 series 2 analyzer using EDTA as a standard.

3.3. M.Wt. determination of grafted polyacrylamide

Approximately 100 mg of graft copolymer was dispersed in 50 ml of deionized water at 80 °C then cooled to below 40 °C. Amyloglucosidase (0.01 ml, 32 U) and α-amylase (0.1 ml, 300 U) were added, and the mixture was digested overnight in incubating shaker (New Brunswick) at 37 °C. Since elution times for the enzymes and glucose were significantly greater than those of the PAAm, digested solutions were used without purification for size exclusion.

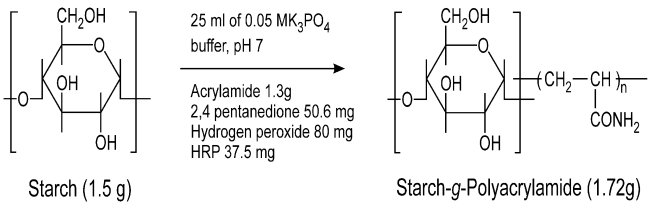
4. Conclusions

In conclusion, starch–polyacrylamide graft copolymers were synthesized by the enzymatic oxidative polymerization of acrylamide and starch using HRP catalyst, H_2O_2 and pentanedione in water and acetate buffer. Further studies to compare the structure and properties of HRP catalyzed vs. conventional persulfate catalyzed graft copolymers are now underway. We are also looking into the mechanism of HRP catalyzed oxidation of starch.

5. Disclaimer on manuscript

- "Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable."

6. Reaction Scheme



Starch (1.5 g) Starch-*g*-Polyacrylamide (1.72g)

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