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Stability of (1 → 3)-β-polyglucuronic acid under various pH and temperature conditions

Erika Watanabe[a], Naoyuki Tamura[a], Shuji Fujisawa[a], Tsuguyuki Saito[a], Naoto Habu[b], Akira Isogai[a,*]

[a] Department of Biomaterial Sciences, The University of Tokyo, Tokyo 113-8657, Japan
[b] Department of Applied Biological Chemistry, Utsunomiya University, Tochigi 321-8505, Japan

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ABSTRACT

Regioselective oxidation of C6 primary hydroxyls to carboxyls was applied to curdlan, to prepare a water-soluble (1 → 3)-β-polyglucuronic acid Na salt [(1,3)-β-PGluA], using a 4-acetamido-TEMPO/NaClO/$NaClO_2$ oxidation system at pH 4.7. Changes in the chemical structure and degree of polymerization of (1,3)-β-PGluA treated in water at various temperatures and pHs were studied to evaluate the stability of (1,3)-β-PGluA to these treatments. This polyuronic acid was found to be stable, without any depolymerization, to treatment in water at pHs 1–9 and room temperature for up to 128 h; slight depolymerization was observed at pHs 11 and 13. When heated in water at pH 1 and high temperatures (1,3)-β-PGluA molecules were randomly depolymerized by hydrolysis, primarily forming glucuronic acid. In contrast, dicarboxylic-acid-type monomers containing ethylene carbons were formed from the C1-carboxyl or C1 reducing ends of (1,3)-β-PGluA molecules by treatment under alkaline conditions; this was initiated by β-alkoxy elimination, similar to the peeling-off reaction of cellulose.

1. Introduction

Curdlan, a linear (1 → 3)-β-glucan, is an extracellular bacterial polysaccharide, and is insoluble in water at room temperature (Harada, Misaki, & Saito, 1968; McIntosh, Stone, & Stanisich, 2005; Stasinopoulos, Fisher, Stone, & Stanisich, 1999). Because of its triple-helical structure, curdlan has peculiar functionalities. Much research has been carried out on curdlan based on its unique structure and functionalities (Chuah, Sarko, Deslandes, & Marchessault, 1983; Deslandes, Marchessault, & Sarko, 1980; Shibakami, Sohma, & Hayashi, 2012). Aqueous suspensions of curdlan form two types of gel, namely low-setting and high-setting gels, on heating, depending on the temperature. Curdlan has therefore been used as an ingredient in various types of processed foods for many years (Fan et al., 2006; Funami, Funami, Yada, & Nakao, 1999; Konno & Harada, 1991; Maeda, Saito, Masada, Misaki, & Harada, 1967). In addition, curdlan has some bioactivities such as immune modulation and antitumor effects, so its derivatives have been used as anticoagulant, antithrombotic, and anti-HIV agents (Bohn & BeMiller, 1995; Jagodzinski et al., 1994; Kataoka, Muta, Yamazaki, & Takeshige, 2002; Ooi & Liu, 2000; Toida, Chaidedgumjorn, & Linhardt, 2003).

Oxidation of the C6 primary hydroxyl groups of polysaccharides using the 2,2,6,6-tetramethylpiperidin-1-oxyl radical (TEMPO), or an analog, as a catalyst under aqueous conditions has opened up a new field of polysaccharide chemistry. The TEMPO-mediated oxidation of polysaccharides has some advantages such as high reaction rates, high regioselectivity, and environmental compatibility (Bragd, Besemer, & van Bekkum, 2001; de Nooy, Besemer, & van Bekkum, 1995). The TEMPO/NaBr/NaClO oxidation system has been applied to various polysaccharides such as cellulose, starch, chitin, and regenerated cellulose (Isogai & Kato, 1998; Kato, Kaminaga, Matsuo, & Isogai, 2004; Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999).

When curdlan was subjected to TEMPO/NaBr/NaClO oxidation in water at pH 10, water-soluble (1 → 3)-β-polyglucuronic acids [(1,3)-β-PGluA] were obtained quantitatively within 100 min. The C6 primary hydroxyl groups of curdlan were mostly converted to carboxylate groups by the oxidation. However, significant depolymerization was inevitable during the oxidation (Tamura, Wada, & Isogai, 2009). In contrast, when curdlan was subjected to oxidation using a 4-acetamido-TEMPO/NaClO/$NaClO_2$ system in water at pH 4.7, completely C6-carboxylated (1,3)-β-PGluA samples with high degrees of polymerization (DPs) were obtained quantitatively (Watanabe, Tamura, Saito, Habu, & Isogai, 2013). Because water-soluble polysaccharides, which originally have triple-helical structures, can be used as effective dispersants of carbon nanotubes and metal nanoparticles in water (1,3)-β-PGluA is also expected to

* Corresponding author. Tel.: +81 3 5841 5538; fax: +81 3 5841 5269.
E-mail address: aisogai@mail.ecc.u-tokyo.ac.jp (A. Isogai).

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have applications as a dispersant (Le et al., 2011; Li, Zhang, Xu, & Zhang, 2011). Investigation of the stability of (1,3)-β-PGluA in water under various temperature and pH conditions is therefore of significance for understanding the fundamental properties and applications of (1,3)-β-PGluA.

In this study (1,3)-β-PGluA samples prepared using the above method were treated in water under various temperature and pH conditions for up to 128 h. The molecular weight parameters and chemical structures of the treated products were analyzed using size-exclusion chromatography with multi-angle laser light-scattering (SEC-MALLS) and photodiode-array detectors and ^{13}C nuclear magnetic resonance (NMR) spectroscopy of the treated products was used to evaluate the stability of (1,3)-β-PGluA. The depolymerization rate constants of this polyuronic acid in water under various conditions were obtained and compared with those of cellouronic acid (Fujisawa, Isogai, & Isogai, 2010).

2. Materials and methods

2.1. Materials

Curdlan was a commercial product (Wako Pure Chemicals Industries Ltd., Japan). 4-Acetamide-TEMPO, sodium chloride, 80% sodium chlorite, 12% sodium hypochlorite solution, and other reagents and solvents were of laboratory grade and used without further purification. Distilled water of HPLC grade was purchased from Wako Pure Chemicals.

2.2. TEMPO-mediated oxidation of curdlan

Curdlan (1 g, 6 mmol C6-OH) was placed in an Erlenmeyer flask with a magnetic stirring bar. Acetate buffer (0.2 M) at pH 4.7 (100 mL) containing $NaClO_2$ (80%, 0.68 g, 6 mmol) and 4-acetamido-TEMPO (0.096 g, 0.45 mmol) was added to the flask. NaClO solution (0.62 mL, 1 mmol) was added at once to the curdlan suspension, and the flask was immediately capped with a universal stopper. The mixture was stirred at 35 °C for 24 h. Oxidation was quenched by adding an excess of ethanol (100 mL) to the mixture, and the precipitate formed was collected by centrifugation. The oxidized product was dialyzed with deionized water and freeze-dried. The oxidized curdlan obtained was re-oxidized to completely oxidize the small amount of remaining primary hydroxyls, using the method described above, and a (1,3)-β-PGluA Na salt consisting of only glucuronosyl units was obtained in 85% yield (Watanabe et al., 2013).

2.3. Stability tests under various pH and temperature conditions

(1,3)-β-PGluA Na salt solutions (1%, w/v) in 0.1 M acetate buffer at pH 4, 0.1 M phosphate buffer at pH 7, and 0.1 M bicarbonate buffer at pHs 9 and 11 were prepared. A 1% (w/v) (1,3)-β-PGluA solution at pH 13 was prepared by adding appropriate amounts of 0.2 M NaOH and water to a 2% (w/v) (1,3)-β-PGluA/water solution. To the 2% (w/v) (1,3)-β-PGluA Na salt/water solution, 0.2 M HCl was added to prepare a 1% (w/v) (1,3)-β-PGluA suspension at pH 1. When the pH of the (1,3)-β-PGluA Na salt solution was adjusted to 1, a white precipitate was formed in the solution as a result of conversion of the (1,3)-β-PGluA Na salt to (1,3)-β-PGluA with protonated carboxyl groups. Each (1,3)-β-PGluA solution or suspension was put in a vial and shaken at room temperature (25 °C) for up to 128 h. In the case of heating above room temperature, each solution or suspension was put in a test-tube with a Teflon-sealed screw cap, and heated in a block-type heating apparatus for the test-tubes (DTU-1C TAITEC, Japan) at 40–105 °C. After treatment, the sample solution or suspension was neutralized with dilute HCl or NaOH solution. These samples were immediately frozen with liquid nitrogen to stop further structural changes, and kept at −20 °C until use. After defrosting at room temperature, the sample solutions were diluted with 0.1 M NaCl. The molecular weight parameters of the products were determined using SEC-MALLS.

2.4. SEC-MALLS analysis

The sample solution was diluted with 0.1 M NaCl to a concentration of 0.1%. The solutions were subjected to SEC-MALLS analysis (DAWN EOS, λ 690 nm; Wyatt Technologies, USA) using one of the following SEC columns for aqueous systems: SB-806MHQ and SB-802.5HQ, 8 mm $\varphi \times$ 30 cm, Shodex, Japan. A 0.1 M NaCl solution was used as the eluent for measuring the molecular weight parameters of the products. The eluent and sample solutions were filtered using 0.2-μm poly(tetrafluoroethylene) membranes (Millipore, USA) before the SEC-MALLS analysis. The weight- and number-average molecular-weights of the samples were calculated from the SEC-MALLS data using ASTRA software (Wyatt Technologies, USA), with a specific refractive index increment (dn/dc) value of 0.158 mL g^{-1} for (1,3)-β-PGluA (Watanabe et al., 2013). A pullulan standard (weight-average molecular-weight 22,800; Shodex, Japan) was exclusively used to normalize the MALLS photodetectors (ASTRA for Windows user's guide, version 4.90). Details of the SEC-MALLS system used and operating conditions have been described elsewhere (Isogai, Yanagisawa, & Isogai, 2009; Tamura, Hirota, Saito, & Isogai, 2010). The DPs of the (1,3)-β-PGluA-related products were obtained by SEC-MALLS analysis; it was assumed that the products had the same structures as that of the original (1,3)-β-PGluA but with different molecular weights.

2.5. Other analysis

After freeze-drying the sample solutions, D_2O containing 3-trimethylsilyl-2,2,3,3-d_4-propionic acid sodium salt (Aldrich, USA) was added to the samples. ^{13}C NMR spectra of the solutions were recorded on an ALPHA-500 (JEOL, Japan). The data accumulation times of the ^{13}C NMR spectra were approximately 25,000. The carboxyl contents of the samples were determined by electric conductivity titration using 0.05 M NaOH (Saito & Isogai, 2004).

3. Results and discussion

3.1. Stability of (1,3)-β-PGluA at room temperature under various pH conditions

The (1,3)-β-PGluA Na salt was treated in water at pHs 1–13 and room temperature for 0–128 h to evaluate its stability under various pH conditions. Fig. 1 shows the relationships between the treatment time and weight-average DP (DP_w) of the products. When (1,3)-β-PGluA was treated in water at pHs 1–9 for 0–128 h, the DP_w values were mostly unchanged and almost the same as the original value. Hence (1,3)-β-PGluA was stable without any depolymerization in water at room temperature under these pH conditions. Slight decreases in the DP_w values with increasing treatment time were observed when (1,3)-β-PGluA was treated under alkaline conditions at pH 11 or 13; the DP_w values decreased from 300 to 220 and 140 at pHs 11 and 13, respectively, after treatment for 128 h. Thus, the higher the treatment pH, the lower the DP_w value of the products obtained by treatment in these pH regions, even at room temperature.

Fig. 2 shows the changes in the SEC elution pattern of the products obtained from (1,3)-β-PGluA by treatment in water at pHs 1, 9, 11, and 13 and room temperature for 0–128 h. When (1,3)-β-PGluA was treated at pHs 1–7, no shifts in the peak-top positions of the SEC elution patterns were observed. (1,3)-β-PGluA was therefore