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Double helix formation from non-natural amylose analog polysaccharides

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ABSTRACT

Double helix formation from the non-natural anionic and cationic amylose analog polysaccharides (amylouronic acid and amylosamine, respectively) was achieved through electrostatic interactions. A water-insoluble complex was obtained by simply mixing the two polysaccharides in water. The ^{1}H NMR analysis indicated that the formation of the complexes with an approximately equimolar unit ratio from the two polysaccharides was resulted regardless of feed ratios for mixing. The powder X-ray diffraction (XRD) measurement suggested that the helix had larger sizes both in diameter and pitch compared with well-known amylose double helix. The formation of the double helical structure was also examined by theoretical calculations. The double helix models, differing in a chain polarity and a charge state of the residues, were constructed based on the 6-fold left-handed amylose chain of the A-amylose crystal structure. Molecular dynamics calculations indicated that those with an antiparallel chain polarity retained an intertwined form. The antiparallel double helical model with the free form residues was suggested to be the most likely structure for the non-natural polysaccharides.

1. Introduction

Polysaccharides are widely distributed in nature and have important biological functions (Schuerch, 1986). Particularly, their specific functions are controlled by higher-order structures, which are potentially constructed from regular primary structures, such as observed in cellulose and chitin, which have regular structures composed of β(1 → 4)-linked primary units of glucose (Glc) and *N*-acetylglucosamine, respectively. Because of the β-glycosidic arrangement of the unit structures, these polysaccharides can form highly controlled crystalline structures with fibrous alignments, which can function as structural materials in biological systems (Klemm, Heublein, Fink, & Bohn, 2005; Pillai, Paul, & Sharma, 2009). Accordingly, it is typically accepted that the types of glycosidic linkages in polysaccharides strongly affect their higher-order structures. For example, amylose, a component of starch, is composed of Glc repeating units, the same as cellulose, but it does not form the fibrous crystalline structure seen with cellulose, because it is linked through an α(1 → 4)-glycosidic arrangement, the opposite stereoarrangement to cellulose. Because of this stereoarrangement, amylose forms a left-handed helical conformation, spontaneously forming a water-insoluble double-helical assembly (Imberty, Chanzy, Perez, Buleon, & Tran, 1988; Imberty & Perez, 1988). The controlled formation of artificial higher-order structures from non-natural polysaccharides is also a topic of interest in polysaccharide chemistry, because of the potential to design novel compounds with new properties and functions.

The self-assembling properties of amylose families can be changed by modification of the Glc repeating units. For example, amylouronic acid (sodium salt form), an amylose analog polysaccharide composed of α(1 → 4)-linked glucuronic acid (GlcA) repeating units, which is prepared by 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO)-mediated oxidation of amylose (Fig. 1), is water-soluble and does not form a controlled higher-order assembly in water as observed for amylose (Kato, Kaminaga, Matsuo, & Isogai, 2005). We previously synthesized another water-soluble amylose analog polysaccharide, composed of α(1 → 4)-linked glucosamine (GlcN) repeating units (hereafter, named 'amylosamine') by thermostable phosphorylase-catalyzed enzymatic polymerization of α-D-glucosamine 1-phosphate (GlcN-1-P) as the monomer (Fig. 1) (Kadokawa, Shimohigoshi, Yamashita, & Yamamoto, 2015). Enzymatic approaches are well accepted as useful tools for the synthesis of well-defined polysaccharides (Shoda, Uyama, Kadokawa, Kimura, & Kobayashi, 2016). In particular, phosphorylase has been used as an enzyme to practically synthesize polysaccharides (Kadokawa, 2011b, 2016; Kadokawa & Kaneko, 2013; O'Neill & Field, 2015). Phosphorylase catalyzes the enzymatic polymerization of α-D-glucose 1-phosphate (Glc-1-P) as a monomer from maltooligosaccharide as a primer according to the following reversible reaction; $(\alpha(1 \rightarrow 4)\text{-Glc})_n + \text{Glc-1-P} \rightleftarrows (\alpha(1 \rightarrow 4)\text{-Glc})_{n+1} + \text{Pi}$

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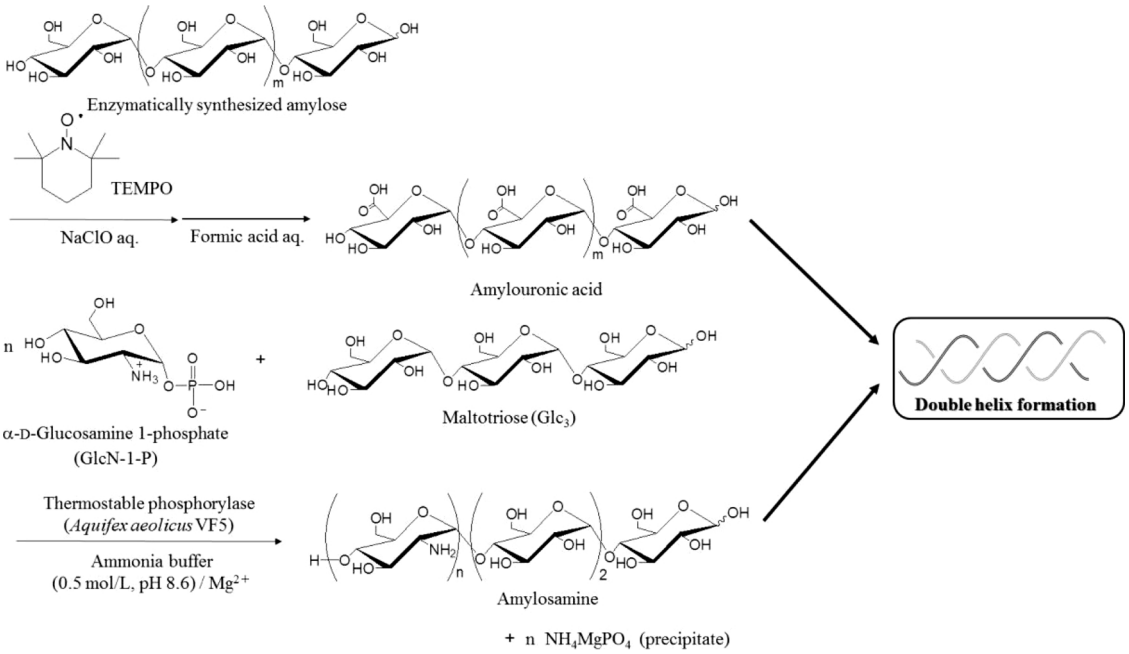

Fig. 1. Synthesis and complex formation of amylouronic acid and amylosamine.

(inorganic phosphate) (Fujii et al., 2003; Ohdan, Fujii, Yanase, Takaha, & Kuriki, 2006; Yanase, Takaha, & Kuriki, 2006; Ziegast & Pfannemüller, 1987). Because phosphorylases show some weak specificity for the recognition of substrates, depending on the source (Kadoakwa, 2015; Kadokawa, 2011a, 2013, 2017), we found that phosphorylase isolated from thermophilic bacteria (*Aquifex aeolicus* VF5) could catalyze the enzymatic polymerization of GlcN-1-P from maltotriose (Glc_3) as a primer. As Pi, which is produced from GlcN-1-P with the progress of the reaction, is a substrate for depolymerization (the reverse reaction in the above scheme), the enzymatic polymerization could be accelerated by removing Pi as an ammonium magnesium precipitate from a reaction medum that contained ammonia buffer and Mg^{2+} to obtain amylosamine. The produced amylosamine was also water-soluble and did not form a controlled higher-order assembly in water.

In this study, we have attempted to construct controlled higher-order assemblies from non-natural anionic and cationic amylose analog polysaccharides through electrostatic interactions (Fig. 1). Consequently, the analytical results suggest the formation of an artificial double helix could be achieved by simply mixing the two materials in water. To confirm formation of a double-helical structure, molecular modeling and molecular dynamics calculations were also conducted for an assembly of the two polysaccharides.

2. Experimental part

2.1. Materials and methods

A monomer, GlcN-1-P, was synthesized by reaction steps in Scheme S1 from D-glucosamine hydrochloride (Nacalai Tesque Inc., Kyoto, Japan) according to the literature procedure (Takata, Shimohigoshi, Yamamoto, & Kadokawa, 2014). Amylose was prepared by the thermostable phosphorylase-catalyzed enzymatic polymerization using Glc-1-P and maltotriose as monomer and primer (feed ratio = 15:1), respectively, at 40 °C for 24 h in 0.2 mol/L acetate buffer (pH 6.2) as described in the literature (Kadokawa et al., 2015). The product was isolated by dialysis against water (molecular cut off: 1000). The average value of degree of polymerization was calculated by the integrated ratio of H-1 signals of repeating unit at 5.1 ppm and reducing end at 4.2 and 4.8 (α and β, respectively) to be 14, which corresponded to M_n = 2290. Thermostable phosphorylase from *Aquifex aeolicus* VF5 (Bhuiyan, Rus'd, Kitaoka, & Hayashi, 2003) was kindly supplied from Ezaki Glico Co. Ltd., Osaka, Japan. Other reagents and solvents were used as commercially received.

^{1}H NMR spectra were recorded on JEOL ECA600 and ECX400 spectrometers (JEOL, Tokyo, Japan). The XRD measurement was conducted using a PANalytical X'Pert Pro MPD (PANalytical B.V., The Netherlands) with Ni-filtered CuKα radiation (λ = 0.15418 nm).

2.2. Computational methods

The structure optimization and molecular dynamics calculations were performed using the PMEMD and PMEMD.CUDA modules of the Amber 14 package (Case et al., 2014) with NVIDIA® Kepler GPUs system (NVIDIA Tesla K20 manufactured and distributed by ELSA Japan Inc). The force-field parameters of the three types of constituent residues, ionic GlcA, and ionic and free GlcN, were obtained from the standard GLYCAM06j-1 parameter set (Basma, Sundara, Calgan, Vernali, & Woods, 2001; Kirschner & Woods, 2001; Kirschner et al., 2008) and the extended set (Singh et al., 2016). The parameters for free GlcA were developed by modifying the GLYCAM06j-1 parameter set in which the atomic charges of the residues were defined according to the RESP procedure (Cornell, Cieplak, Bayly, & Kollman, 1993) using Gaussian 09 (Frisch et al., 2010). The solvated double helix model systems with TIP3P water models (Jorgensen, Chandrasekhar, Madura, Impey, & Klein, 1983) were optimized and heated from 100 K to 300 K in the NTV ensemble followed by the NTP production dynamics at 300 K and 1 bar for 20 or 50 ns. The SHAKE option (Ryckaert, Ciccotti, & Berendsen, 1977) was adopted with a 2 fs time step to constrain the motions of the hydrogen atoms. The torsional angles of the O5−C1−C2−C3 and C3−C4−C5−O5 bonds of the pyranose rings were also constrained to avoid unrealistic deviation from the $^4C_1$ chair form of the original residues.

2.3. Synthesis of amylouronic acid

A solution of 11% NaClO aqueous solution (0.80 mL) was added to a solution of an enzymatically synthesized amylose (0.050 g, 0.0234 mmol) and TEMPO (0.023 g, 0.0147 mmol) in water (1.5 mL) at 0 °C. After the mixture was stirred at that temperature for 4 h, it was poured