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New insights into the action mode of amylosucrase on amylopectin

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ABSTRACT

To investigate the action mode of amylosucrase (AS) on amylopectin, waxy corn starch (WCS) was selected as an acceptor. The effects of WCS dissolution method, reaction temperature, sucrose concentration and AS activity on transglycosylation degree (TD) were investigated. Under the selected reaction condition, the enzymatic reaction process was divided into two stages, i.e. before and after 0.25 h, of which the relations between TD value and reaction time were successfully described using a linear and a logarithmic function, respectively. Then, the elongated WCSs with different TDs were produced according to the theoretical reaction time calculated based on the regression equations. The chain length distribution of the elongated WCSs indicated that all of the branch chains of WCS were greatly elongated by AS before occurrence of starch precipitation. Afterwards, however, AS merely elongated the branch chains whose non-reducing ends were exposed on the surface of the precipitate.

1. Introduction

Amylosucrase (AS) from *Neisseria polysaccharea* is a glucosyltransferase belonging to hydrolase family 13 [1–3], and numerous studies have investigated the catalytic properties of AS [2,4,5]. When using sucrose as a sole substrate, it catalyzes the transglycosylation reaction and produces the oligosaccharide in addition to the synthesis of amylose-like polymers. Besides, in the presence of a primer glucan, AS catalyzes the transfer of a D-glucose residue typically obtained from sucrose onto the non-reducing end of preexisting acceptor, releasing fructose [6]. Previously, the action of AS on glycogen has been investigated, and the results showed that the elongation occurred on some branched chains of the glycogen molecule [2]. Furthermore, a non-homogeneous multigrafting mechanism had been suggested by Rolland-Sabaté et al. [6] when a large series of branched polymers were used as acceptors, including glycogen, phytoglycogen, amylopectin, and limit dextrins. They concluded that AS randomly elongated some external chains of those acceptors. However, they failed to analyze the branch chain length distribution of the elongated polymers since precipitation occurred during debranching. As it is known, elongation of the branched polymers is bound to induce precipitation [6,7]. And to the best of our knowledge, no literature has reported the effect of reaction product precipitating process on the action of AS on branched polymers.

In this study, waxy corn starch (WCS) was selected as an acceptor, and elongated WCSs with different transglycosylation degrees (TDs) were prepared by controlling the enzymatic reaction time. To determine the action mode of AS on acceptor, the chain length distributions of the elongated WCS were analyzed by high-performance size-exclusion chromatography (HPSEC).

2. Materials and methods

2.1. Materials

WCS was obtained from Ingredion Inc. (Westchester, IL, USA). Sucrose and fructose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). Isoamylase (1000 U/mL) was purchased from Megazyme (Wicklow, Ireland). Other chemicals and reagents were all analytical grade.

2.2. Preparation of AS

The synthesis of *N. polysaccharea* amylosucrase gene (GenBank: AJ011781.1) was performed by Sangon Biotech (Shanghai, China). The company provided us with the gene cloned into the

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pRSET-B vector. The constructed plasmid was transformed into *Escherichia coli* BL21, and subsequently induced by isopropyl-β-d-thiogalactoside (IPTG) to express amylosucrase protein. The recombinant amylosucrase was purified as previously described [8]. Enzyme activity was measured using the method proposed by Ryu et al. [9].

2.3. Transglycosylation reaction

Transglycosylation reaction was carried out in an enzymatic reactor with a total volume of 100 mL and a final WCS concentration of 1.0% (w/v). The reaction mixture was stirred using a mechanical overhead stirrer (RW 20, IKA, Germany) at a speed of 200 rpm. Before adding the enzyme, WCS solution (1.25%, w/v) and sucrose solution (1.0 M, in 50 mM Tris-HCl buffer, pH 7.0) were premixed for 15 min to achieve thermal equilibrium. To investigate the effects of enzymatic reaction conditions on TD, aliquots (0.5 mL) of the reaction mixture were collected at prescribed time intervals and heated in a boiling water bath for 10 min to inactivate the enzyme.

2.4. Determination of TD

TD was established with the reference of Rolland-Sabaté et al. [6]. It was defined as the percent of grafted glucosyl residues against acceptor in the enzymatic reaction. By determining the amount of fructose released in the reaction system using the dinitrosalicylic acid (DNS) method [10], TD could be calculated as follows:

$$\mathrm{TD}\,(\%)\frac{162 \times m_{fru}}{180 \times m_{wcs}} \times 100$$

where m_{fru} is the weight of fructose, m_{wcs} is the initial weight of WCS, 162 and 180 is the molar weights of glucosyl units and fructose, respectively.

2.5. The effects of enzymatic reaction conditions on TD

To investigate the effect of WCS dissolution method on TD, the WCS was dissolved in 50 mM Tris-HCl buffer (pH 7.0) (1.25%, w/v) using three methods. (a) Boiling: the suspension was heated in a boiling water bath with constant magnetic stirring for 1 h; (b) autoclaving: the suspension was autoclaved at 121 °C for 15 min; (c) DMSO pretreatment: the WCS was pretreated by dimethyl sulfoxide (DMSO) as previously described [11]. The effects of reaction temperature, sucrose concentration and AS activity on TD were investigated within the range of 30–40 °C, 0.10–0.20 M, and 1400–2800 U/L, respectively.

2.6. Turbidimetry

Under the selected reaction condition, the turbidimetry of the reaction mixture was analyzed with the following method of Potocki-Veronese et al. [12] with slight modifications. At prescribed time intervals, 1 mL of the reaction mixture was collected and transferred into a colorimetric utensil immediately. The absorbance was determined at 600 nm, using deionized water as the counterpart.

2.7. Preparation of elongated WCS

Under the selected reaction condition, the process of the reaction was simulated by mathematical models. The elongated WCSs with different TDs (40, 80, 120, 160, and 200%) were prepared according to the theoretical reaction time which was calculated based on the regression equations. After transglycosylation reaction, the reaction mixture was heated in a boiling water bath to inactivate the enzyme, cooled to room temperature and stored in a refrigerator at 4 °C overnight. The supernatant was discarded after centrifugation (12, 000*g*, 20 min), while the insoluble fraction was washed 5 times with distilled water, then freeze-dried and ground to pass through a 200-mesh sieve.

2.8. Determination of branch chain length distribution by HPSEC

The branch chain length distributions of the native and elongated WCSs were analyzed by HPSEC, using a previously described process [13] with modifications. To avoid linear chain precipitating during debranching, the concentration of starting starch solution was decreased to 0.5 mg/mL, and the temperature of debranching reaction was increased to 50 °C. Once the temperature of the gelatinized starch solution decreased to 50 °C, isoamylase (2 μL) was immediately added. Shodex OHpak SB-804HQ and 802.5HQ (Showa Denko, Tokyo, Japan) columns were connected in this order and maintained at 50 °C. Shodex pullulan P-82 (Showa Denko, Tokyo, Japan), maltopentaose and maltoheptaose (Hayashibara Biochemical, Okayama, Japan) were employed as standards. The molecular weights (M_w) of branch chains were calculated from the standard cure. Values of the chain lengths, expressed as degree of polymerization (DP), were thereafter calculated using the following equation:

$$\mathrm{DP} = M_w/162$$

where 162 is the molar weight of glucosyl units.

2.9. Statistical analysis

The experiment was performed in duplicate, and analyses were conducted in triplicate. Values were expressed as the mean ± SD.

3. Results and discussion

3.1. The effects of enzymatic reaction conditions on TD

The effect of WCS dissolution method on TD was investigated at 35 °C with 0.10 M sucrose and a fixed enzyme activity of 1400 U/L. Complete dissolution of WCS may facilitate enzymatic reaction. However, no significant difference in TD values was observed among the three dissolution methods after 24-h reaction (Fig. 1a). Thus, the method of boiling for 1 h was selected for convenient operation. Since the reported optimal temperature of AS varied between 30 °C and 42.5 °C [14–16], the effect of reaction temperature on TD was investigated within the range of 30–40 °C in the present study. Fig. 1b showed that the reaction temperature had considerable influence on TD. Much higher TD values were observed at 35 and 40 °C than at 30 °C. Considering higher temperature may result in the inactivation of AS, 35 °C was selected as the reaction temperature. The effect of sucrose concentration on TD was investigated at 35 °C with a fixed enzyme activity of 1400 U/L. As shown in Fig. 1c, during the initial 4-h reaction, there was no significant difference in TD values among the three levels of sucrose concentration investigated, indicating that sufficient substrate existed in all of the three reaction systems. However, after 24-h reaction, the TD values gradually increased from 168% to 216%, along with the increase in sucrose concentration from 0.1 to 0.2 M. To obtain the elongated WCS with higher TD, 0.2 M sucrose was chosen and applied to further study. Enzyme activity is a key factor of an enzymatic reaction. In the present study, the effect of enzyme activity on TD was investigated within the range of 1400–2800 U/L. As shown in Fig. 1d, the TD values gradually increased as the AS activity increased from 1400 to 2800 U/L. However, there was no definite multiple relationship between the TD value and AS activity level. In order to shorten the reaction time and thus minimize the