Irradiation of Ultrasound onto Substrate Mixture Enhances Transglycosylating Activity of Commercial α-Amylase Preparation

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Irradiation of ultrasound onto a mixture of starch and *n*-octyl β-D-glucopyranoside enhanced the transglycosylating activity of commercial α-amylase preparation, producing *n*-octyl β-D-isomaltoside in good yield. The enhancement was attributable to the formation of an inclusion complex with the glycosyl donor and the glycosyl acceptor.

There is high current interest in developing synthetic routes for obtaining oligosaccharides.[1] Enzymatic transglycosylation is an attractive technique for preparing bioactive and functional glycomaterials, e.g. glycolipids and glycopeptides.[2,3] There have been numerous trials to increase the efficiency of the enzymatic reactions. The modification of the active center of glycosidases by site-directed mutagenesis changes the transglycosylation activity of the enzymes.[4,5] Engineering of reaction media, such as organic solvents and supercritical fluids, is also applicable, and several methods by using enzymatic transglycosylation reactions in homogeneous organic media have been reported.[6–8]

α-Amylases are widely occurring monomeric enzymes that catalyze the hydrolysis of the internal α-(1,4) glucosidic linkages in starch, releasing α-anomeric products. These enzymes are also known to catalyze transglycosylation reactions with various acceptors.[9–12] However, glycolipids were not acceptable by α-amylases because the reactions take place in heterogeneous media, which cannot dissolve glycosyl donor and glycosyl acceptor simultaneously.

It is well known that starch (including amylopectin and amylose) forms inclusion complexes with hydrophobic fatty acid and adsorbs hydrophobic alcohols.[13–15] The use of such inclusion complexes is ideal, because proximity of glycosyl donor and acceptor in active site of enzyme is able to satisfy an efficient transglycosylation reaction.

In this paper, we report that irradiation of ultrasound onto substrate mixture (starch as a glycosyl donor and *n*-octyl β-D-glucopyranoside as a glycosyl acceptor) enhanced transglycosylation activity of commercial α-amylase preparation from *Aspergillus oryzae*. We have chosen ultrasound irradiation in order to facilitate the formation of complexes of donor and acceptor in heterogeneous media.

Commercial α-amylase preparation (Biozyme A, 90,000 U/g) was a kind gift from Amano Enzymes (Nagoya, Japan). The glycosyl donor of a soluble starch was prepared by treating soluble starch (Nakalai Tesque, Tokyo) by pullulanase. The glycosyl acceptor of *n*-octyl β-D-glucopyranoside (C_8Glc) was obtained from Nakalai Tesque (Tokyo, Japan).

The transglycosylating activity was evaluated as follows: A suspension of 1.0 g of pullulanse-treated soluble starch and 10 mg of C_8Glc in 50 mM sodium acetate buffer (pH 5.0, 50 mL) was ultrasound-irradiated by Branson 250 Sonifier (20 kHz, 25 W, 30.5 °C, Branson Corp., USA) for 30 min. To this mixture, a solution of α-amylase (2.4 mg) in acetate buffer (pH 5.0) was added and the reaction mixture was incubated at 40 °C. The resultant products were separated by reversed phase high performance liquid chromatography. The structure of main glycosylation product, *n*-octyl β-D-isomaltoside (C_8IMal) was determined by ^{1}H, ^{13}C, HMBC, and HMQC NMR and MALDI-TOF MS analysis.

Figure 1 shows the time course of the products conversion by using the ultrasound-irradiated substrates (●) and simple mixture of starch and C_8Glc (■). The yield of the transglycosylation product reached 60% after a day, indicating that transglycosylating activity is 2.5-fold higher than that of simple mixing of the substrates. When the simple mixture was employed, only a trace amount of the transglycosylation products were identified after 30 min from the initiation, probably due to the difficulty of forming glycosyl donor and acceptor complexes.

Figure 2 shows the effect of concentration of soluble starch on reaction rate. The initial rate of transglycosylation reaction under the lower concentration (1.5%) was much faster than that under higher concentration (3.0%). However, the yield of the transglycosylation reaction reached a plateau after 2 h (up to 30% conversion), because all soluble starch was consumed. On the contrary, the yield of the product under higher glycosyl donor concentration increased as the reaction proceeded (up to 60%). The suppression of the reaction rate at the initial stage of the reaction (≈1 h) may be caused by higher viscosity of the reaction media.[16,17]

In order to evaluate the supramolecular formation of starch

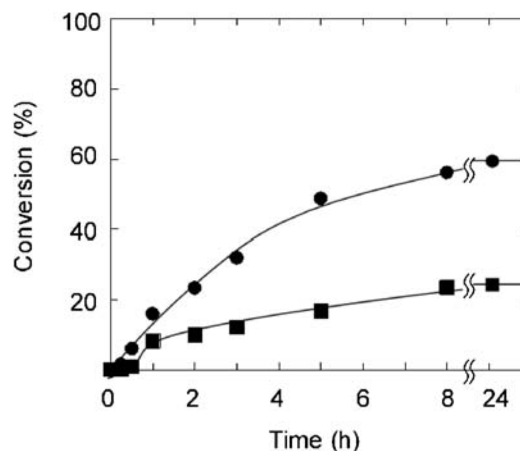

Figure 1. Typical time course of the transglycosylation reaction from soluble starch to C_8Glc catalyzed by α-amylase. Enzymatic reactions were carried out by using ultrasound-irradiated substrate mixture (●) or simple mixture of the substrates (■).

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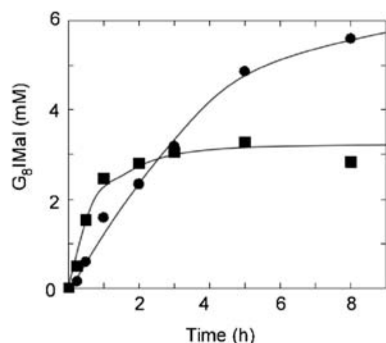

Figure 2. Effect of soluble starch concentration on the transglycosylation efficiency. ● 3.0% (w/v) and ■ 1.5% (w/v) of soluble starch.

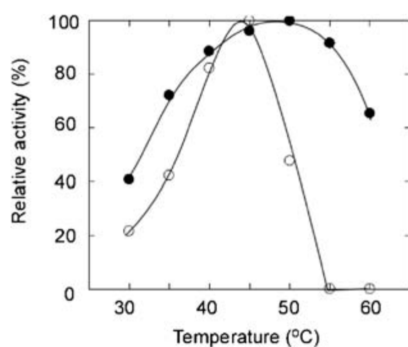

Figure 3. Effect of reaction temperature on transglycosylation reaction and hydrolytic reaction. ○ Transglycosylation reaction; ● Hydrolytic reaction.

and C_8Glc, the effect of reaction temperature was examined (Figure 3). The optimal temperature for the transglycosylating activity was around 45 °C and the activity decreased as the temperature increased (○ in Figure 3). Interestingly, no transglycosylating activity was observed above 55 °C, where the catalyst α-amylase still retains its hydrolytic activity (ca. 90% activity at 55 °C, ● in Figure 3). These results clearly indicate that the disappearance of the transglycosylating ability above 55 °C is not attributed to the inactivation of the enzyme by heating. The proximity of glycosyl donor and glycosyl acceptor cannot be realized, because the supramolecular complex composed by starch and C_8Glc may be destroyed over 55 °C.

The mechanism of the octyl disaccharide formation consists of the initial formation of a starch–C_8Glc complex followed by α-amylase-catalyzed glycosyl transfer from glycosyl donor (starch) to *n*-octyl glucoside, which is in the proximity of the starch molecule (Figure 4). According to α-amylase reaction mechanism, these final products were difficult to be obtained directly, because α-amylase can not catalyze glucosyl-transferring reaction. In order to clarify this discrepancy, MALDI-TOF MS analysis of reaction product in early stage of the reaction was performed. The molecular ions of *n*-octyl β-D-maltooligosides (up to maltoheptaoside) were detected, indicating that α-amylase catalyzed maltooligosyl-transferring reaction onto C_8Glc and trimmed until C_8IMal was formed.[18]

In conclusion, the idea using inclusion complex-forming ability of starch molecules as substrates for transglycosylation reaction provides us a novel technique for facile glucosyl-transferring reaction on glycolipids having low solubility in aqueous solution. This is the first report of α-amylase-catalyzed synthesis of glycolipid irrespective of the use of ultrasound-irradiated substrate mixtures. Work on precise reaction mechanism is currently in progress.

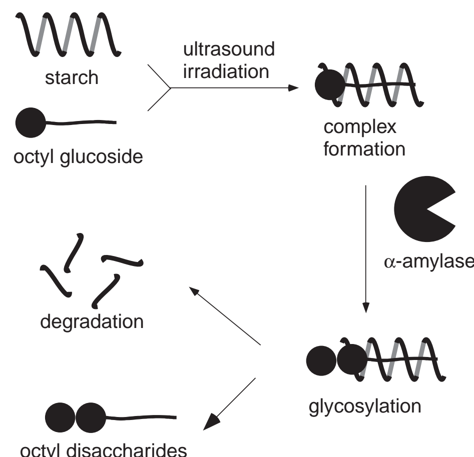

Figure 4. Proposed mechanism of α-amylase-catalyzed transglycosylation reaction employing complexed substrates.

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References and Notes

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18 The possibility of the participation of α-glucosidase contained in the α-amylase preparation cannot be ruled out.

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