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Heterooligosaccharide synthesis catalyzed by α-glucosidase from *Bacillus stearothermophilus*

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Abstract

α-Glucosidase from *Bacillus stearothermophilus* was used as a catalyst for oligosaccharide synthesis by reversed hydrolysis. The yield of disaccharides and trisaccharides depended strongly on the units of enzyme activity added, and on the stability of the enzyme under reaction conditions. When glucose was the only saccharide present in the reaction mixture with α-glucosidase, isomaltose (51%), nigerose (25%), maltose (14%) and kojibiose (10%) were formed. In 50% glucose solution, disaccharide concentrations reached up to 400 mmol/l and trisaccharides were also produced. When other saccharides (mannose or xylose), in addition to glucose, were present in the reaction mixture, both homodisaccharides and heterodisaccharides were formed, their quantity being dependent on the glucose/saccharide acceptor ratios. The highest yields of oligosaccharides were observed with glucose alone, consistent with the observation that the enzyme stability was highest with glucose as the sole saccharide.

Keywords: α-Glucosidase; Reverse hydrolysis; Heterooligosaccharide synthesis

1. Introduction

Recently, much effort has been devoted to studying the reverse reactions of glycosidases as a means of preparing oligosaccharides [1,2]. α-Glucosidases are enzymes that hydrolyze terminal non-reducing α-linked glucose residue from short oligosaccharides, releasing D-glucose. They are highly specific for the glucose residue, however; their specificity towards the aglycon portion of the substrate is not as distinct [3]. This suggests that α-glucosidase could be a suitable and useful enzyme for the preparation of various heterooligosaccharides.

This paper describes the synthesis of glucooligosaccharides via condensation of glucose with different saccharide acceptors catalyzed by α-glucosidase from *Bacillus stearothermophilus*.

2. Experimental

2.1. Materials

D-Glucose, D-mannose, D-xylose and *p*-nitrophenyl-α-D-glucopyranoside (PNPG) were purchased from Sigma (USA). Reagents used for structural analysis of products were pur-

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chased from Fluka and Merck (Germany). α-Glucosidase from *B. stearothermophilus* was a commercial product (Sigma, USA).

2.2. Enzyme assay

The α-glucosidase activity was measured using PNPG as a substrate. One unit of the activity was defined as the amount of the enzyme that produces 1 μmol of *p*-nitrophenol per 1 min at pH 6.8 and 37°C. The enzyme was incubated at 37°C with 37 mM PNPG solution in 0.1 M sodium phosphate buffer at pH 6.8 for 15 min. Reaction was terminated by adding 10% Na_2CO_3 (w/v) and liberated *p*-nitrophenol was detected spectrophotometrically at 405 nm.

2.3. Condensation reactions

Reaction mixtures for reverse hydrolysis were 50% (w/w) solution of total carbohydrates, which consisted either of glucose alone, or mixtures (1:1, w/w) of glucose and an acceptor sugar (xylose or mannose) in 0.1 M phosphate buffer pH 7.5. α-Glucosidase was added and the reaction mixture was incubated. Two sets of samples were taken at various time intervals from reaction mixture. The first one was analyzed by high performance liquid chromatography (HPLC) for saccharide composition, the second one was diluted and the remaining enzyme activity was assayed with PNPG as substrate.

2.4. HPLC

Oligosaccharide synthesis was monitored by HPLC (Waters, USA). All reaction products were identified and quantified on Supelcogel Ca column with elution by deionized water at a flow rate of 0.5 ml/min at 80°C. Elution was monitored by a differential refractometer. Product quantification was based on a comparison of peak areas with those of standard sugars and galactose or sorbitol as internal standards.

2.5. Structural analysis of reaction products

To analyze the structure of condensation products, α-glucosidase was incubated in 50% (w/w) glucose solution or in mixture of glucose and mannose (1:1). 1 ml aliquots were taken after 4 days. Products were purified by gel filtration on a column (2.5 × 120 cm) of Biogel P-2 (fine grade) eluted with degassed, deionized water at 0.25 ml/min. Carbohydrates were detected by refraction index. All fractions containing disaccharides were collected and lyophilized prior to structural analysis. Product structures were analyzed by methylation analysis using the methylation protocol of Ciucanu and Kerek [4] and the hydrolysis and reduction protocol of Harris et al. [5]. Partially methylated alditol acetates were analyzed by GC–MS.

2.6. GC–MS

Experiments were performed on a MAGNUM GC–MS ion trap system (Finnigan MAT, USA) equipped with heated inlet option (Spectronex AG, Basel, Switzerland). System utilized Varian SPI injector (held at 125°C and programmed to 250°C at the rate of 125°C/min) and DB-5 ms capillary column (JW Scientic, USA) 30.0 × 0.25 mm with 0.25 μm thickness. The carrier gas (He 99.996%) velocity was 33.1 cm/s (at 60°C). The GC oven was maintained at 100°C for 0.10 min, increased by 3°C/min to 250°C, and held for 5 min at the maximum of 250°C. Samples were introduced as 0.5 μl aliquots with a Hamilton syringe. The transfer line was held at 250°C and ion manifold at 200°C. The ion trap was tuned using default software setting (Magnum 2.4., Finnigham MAT) to obtain suitable mass calibration, filament emission current, multiplier voltage and automatic gain control (AGC) settings. The GC–MS ion abundance test using *p*-fluorobromobenzene and decafluorotriphenylphosphine was performed weekly to GC–MS system performance.

3. Results and discussion

3.1. Condensation reaction in glucose

The effect of four different values of α-glucosidase activities (10, 30, 65 or 130 U/g of reaction mixture) in the course of reverse hydrolysis was studied in 50% (w/w) solution of glucose (Fig. 1).

The rate of disaccharide formation was significantly affected by the enzyme activity used. Maximum obtained disaccharide concentration was 375 mmol/l. Higher yields can be expected after prolonged reaction, as after 10 days, the reaction has not yet reached equilibrium. With the two highest tested enzyme activities, formation of the trisaccharide was also observed as soon as the disaccharide concentration reached 200 mmol/l (e.g. from the second day with the activity of 130 U/g) and their concentration constantly increased. Structure analysis of synthesized products with GC–MS revealed the following: four different linkages are formed during the reaction and their percentages are: (1 → 2)- 10%; (1 → 3)- 25%; (1 → 4)- 14% and (1 → 6)- 51%. While in the direction of hydrolysis, the studied bacterial α-glucosidase is highly specific for (1 → 4)-linkages and catalyzes the hydrolysis of other linkages at considerably lower rates, the main product of reversed reaction is isomaltose with α(1 → 6)-linkage. This suggests that the formation of individual isomers in the synthesis catalyzed by this enzyme is under thermodynamic control. According to results obtained previously [6], tranglycosylation can take place during the reverse reaction and isomaltose and nigerose can also be synthesized in this way.

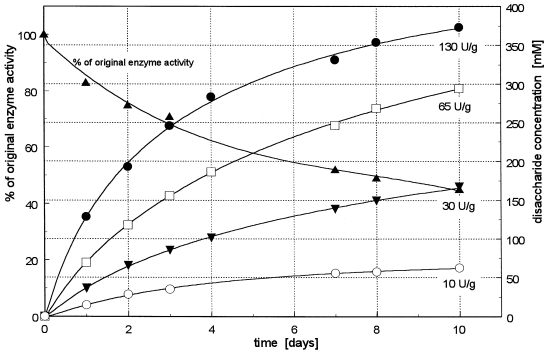

Fig. 1. Disaccharide formation in 50% solution of glucose under different activity of α-glucosidase from *B. stearothermophilus*.

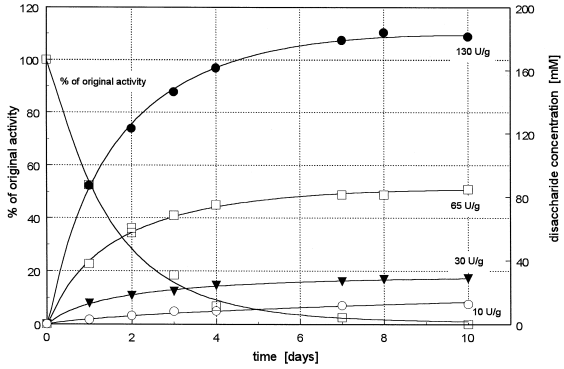

Fig. 2. Disaccharide formation in 50% solution of glucose and mannose (1:1) under different activity of α-glucosidase from *B. stearothermophilus*.

3.2. Condensation reaction in a mixture of glucose and mannose

The reversed reaction with bacterial α-glucosidase was carried out in a 50% solution of a glucose/mannose mixture (1:1) under the same conditions as in the experiment with glucose alone. Results are shown in Fig. 2.

The rate of disaccharide formation for all tested enzyme activities was slower compared with reaction in glucose alone, and was significantly affected by a faster decrease of enzyme activity in this solution. Loss of enzyme activity probably resulted in a reduction of reaction rate and termination of disaccharide synthesis. Structure analysis of products with GC–MS revealed that both homo- and heterodisaccharides are synthesized in the ratio 58%:42% (based on peak areas on GC). Two different heterodisaccharides were formed during the reaction; Glu *p*-(1 → 3)-Man *p* and Glu *p*-(1 → 6)-Man *p*. Among the synthesized homodisaccharides, the same isomers were found in the reaction mixture as during reaction in 50% glucose.

Table 1
Homo- and heterodisaccharide synthesis with the different xylose/glucose ratios

	Homooligosaccharide synthesis [mM]				Heterooligosaccharide synthesis [mM]			
Xylose/glucose (day)	30:70	50:50	80:20	90:10	30:70	50:50	80:20	90:10
1	25	12	5	4	22	24	21	16
2	36	19	8	4	29	36	27	19
3	48	24	11	5	36	43	35	21
4	53	25	10	4	38	46	39	21
5	55	26	11	4	40	48	40	21
7	61	28	10	5	44	53	42	22

3.3. Effect of different ratios of xylose and glucose on reverse hydrolysis

To analyze the effect of different ratios of acceptor and glucose on the reaction rate and enzyme stability, xylose was chosen as an acceptor. In the case of this saccharide, synthesized homo and heterooligosaccharides were well-separated by HPLC and their formation could be evaluated independently (Table 1).

By measuring enzyme activity during experiments (Fig. 3), it was shown that the higher the amount of xylose in the reaction mixture, the faster the decrease of α-glucosidase activity during the experiment. Different enzyme stability and different glucose concentration was reflected in the formation of both types of disaccharides. At highest xylose/glucose ratio (90:10), heterodisaccharides were synthesized as the major products and the amount of homodisaccharides were negligible. However, the final concentration of heterodisaccharides was the lowest from all ratios tested as their synthesis had stopped from the second day, as a consequence of the loss of most of the initial enzyme activity. Increasing the glucose concentration (ratio 30:70) promoted formation of homodisaccharides and improved enzyme stability. Better stability enabled synthesis of heterodisaccharides as well, and they reached higher concentrations in the reaction mixture than those observed with the highest tested acceptor/donor ratios (90:10).

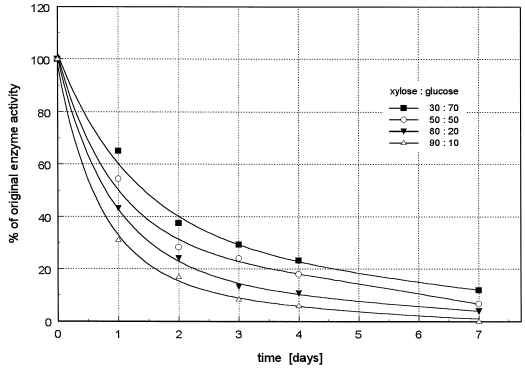

Fig. 3. Loss of the enzyme activity at different xylose/glucose ratios.

Based on these results, we assume that the loss of enzyme activity is not caused by the inhibition effect of synthesized disaccharides. This was confirmed by the observation that fresh enzyme added into the boiled samples taken from reaction mixture was not inhibited.

Synthetic reactions are usually carried out at higher temperatures because of thermal stabilization of the enzyme by the appropriate sugar. To analyze if other sugars (mannose or xylose) have the same effect on this α-glucosidase as glucose, enzyme was incubated in the 50% solutions of mannose or xylose at 55°C. We observed faster loss of enzyme activity with these sugars when compared with the reaction in glucose solution under the same conditions (no disaccharides were analyzed by HPLC analysis of acceptor solutions). This implies that mannose and xylose exhibit insufficient stabilization effects on the enzyme and the measured loss of α-glucosidase activity during the reaction is caused by thermal denaturation of the enzyme.

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