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THE SYNTHESIS OF OLIGOSACCHARIDES BY THE REVERSED HYDROLYSIS REACTION OF β-GLUCOSIDASE AT HIGH SUBSTRATE CONCENTRATION AND AT HIGH TEMPERATURE

Katsumi Ajisaka*, Hiroko Nishida, and Hiroshi Fujimoto

Meiji Institute of Health Science
540 Naruda, Odawara 250, Japan

SUMMARY

In the presence of β-glucosidase from almond, a 90% glucose solution gave four kind of β-linked glucose-disaccharides. The yield increased as the concentration of glucose was increased and as the reaction temperature was raised. The maximum yield of disaccharides from 90% glucose solution was 40% at 55°C.

INTRODUCTION

Many hydrolytic enzymes have successfully been used for the synthesis of peptides (Miranda et al., 1986), lipids (Takahashi et al., 1984), or other esters (Vidaluc et al., 1983) by reversing the normal hydrolytic reaction of the enzyme. However, the synthesis of oligosaccharides by similar method is not practical at present, because the equilibrium of the reaction exists extremely in the hydrolytic direction (Tanaka and Oi, 1986). Very recently, it was reported that good yield of mannose-disaccharides was obtained by the incubation of high concentration of mannose with jack bean α-mannosidase at high temperature (Johansson et al., 1986). This result indicates that the concentration of the substrate is one of the most important factors in order to shift the equilibrium to the direction to yield the disaccharides.

In the present study, the best condition to obtain the high yield of oligosaccharides by reversed hydrolysis reaction was investigated using β-glucosidase from almond

as a model reaction.

MATERIALS AND METHODS

β-Glucosidase from almond was obtained from Sigma Chemical Co., and was purified by DEAE-Toyopearl (Toyo Soda Co., Japan) equilibrated with 20 mM Tris-HCl buffer (pH 8.0). The column was eluted with a linear gradient of NaCl (0-0.5 M). The standard incubation mixture was composed of glucose, 0.1 M phosphate buffer, and enzyme (110 units/ml). Below pH 4.5, 0.1 M acetate buffer was used instead of phosphate buffer. For the incubation at 37 °C, the reaction vessel was rotated with a rotator at an ambient temperature of 37 °C. For the reaction at 55 °C, the solution was incubated in an oil bath at 55 °C and stirred magnetically. Products were analyzed by HPLC (Hitachi 655A-11 fitted with Zorbax-NH_2 column) using a solvent system of acetonitrile-water (70 : 30). Detection of the products was carried out by Shodex SE-51 differential refractometer. The yield of the oligosaccharides was estimated by measuring the weights of the peaks in the HPLC chart. When necessary, the fraction of HPLC was identified by 100 MHz ^{13}C NMR spectra measured with Varian XL-400 NMR spectrometer.

RESULTS AND DISCUSSION

Reaction profile Figure 1 shows a representative HPLC chart of the reaction mixture from 90% (w/v) glucose solution containing β-glucosidase from almond after the incubation for 24 hr at 37 °C. The components in each peaks were identified by comparison of the retention times of authentic standards. When necessary, 100 MHz ^{13}C NMR spectra were measured for further identification. Figure 1 shows that gentiobiose is a major product, and that small amounts of laminaribiose, sophorose, cellobiose, and some trisaccharides are also present. Although cellobiose and sophorose could not be separated by the elution condition used in the present study, ^{13}C NMR spectrum of the fractions of the corresponding peaks clearly exhibited the presence of approximately equal amounts of cellobiose and sophorose (Figure 2). All the disaccharide peaks in the HPLC chart were cut out and weighed for the calculation of the yield. The ratio of gentiobiose : (sophorose + cellobiose) : laminaribiose was 6 : 3 : 1.

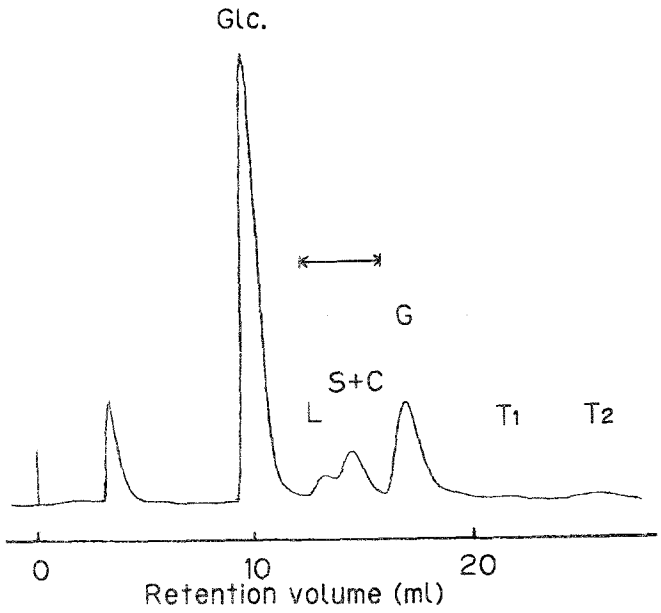

Figure 1 : HPLC after incubation of 90% glucose solution (pH 4.0) in the presence of β- glucosidase(110 units/ml) at 37°C for 24 hr. Peaks Glc, L, S, C, and G correspond to glucose, laminaribiose, sophorose, cellobiose, and gentiobiose, respectively. Peaks T_1 and T_2 are unidentified trisaccharides. The fractions denoted by arrow were collected for a measurement of ^{13}C NMR spectroscopy.

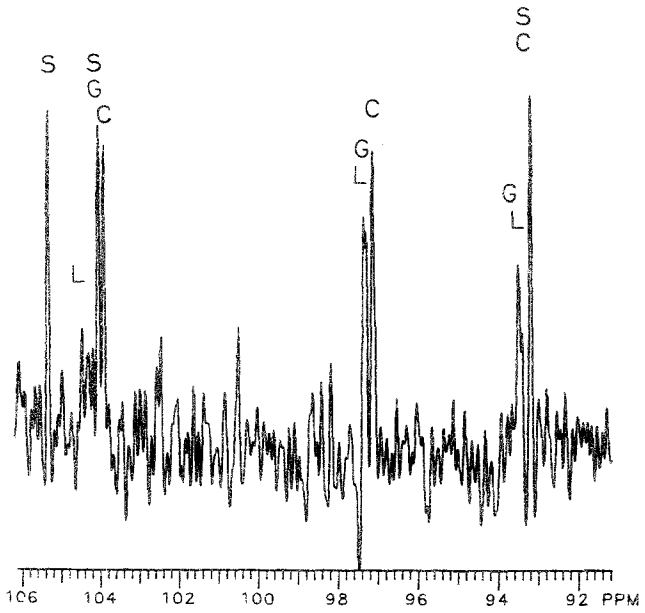

Figure 2 : The region of C-1 carbon signals in 100 MHz ^{13}C NMR spectrum of the fractions denoted in Figure 1. L, S, C, and G are the same as in Figure 1.

pH Dependency To examine the effect of pH on the condensation reaction, 90% (w/v) glucose solution containing β-glucosidase from almond was incubated at 37°C over the range of pH 3.0 to 8.0. The result was shown in Figure 3. It can be recognized from Figure 3 that an optimum pH for the synthesis of disaccharides by the

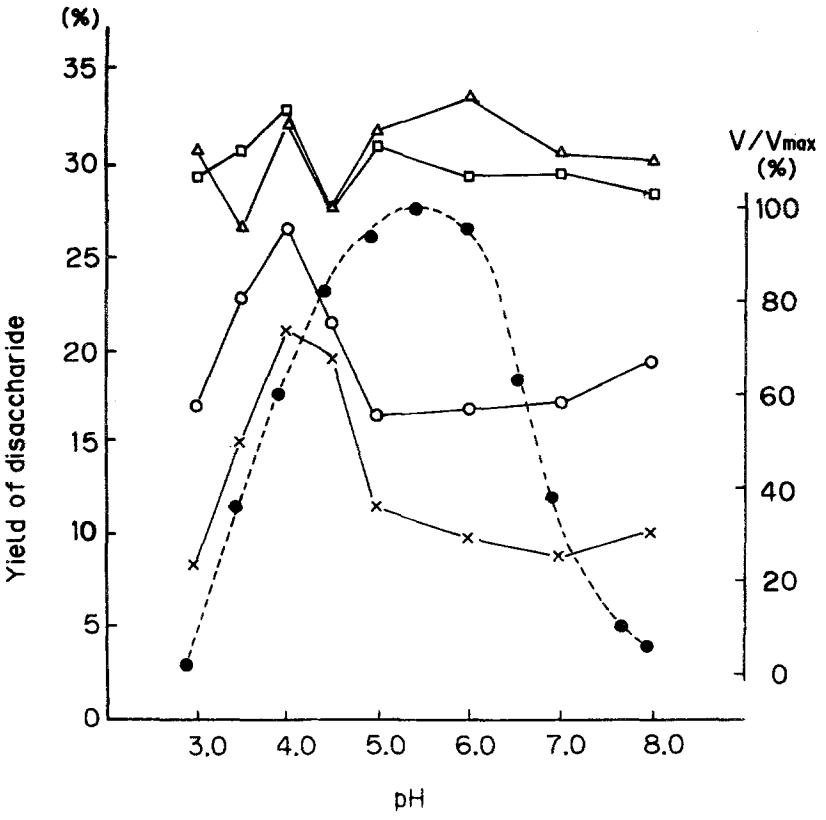

Figure 3 : pH dependence of the formation of disaccharides and the hydrolysis of oNPG. 90% Glucose solution was incubated at 37 °C in the presence of 110 units/ml β-glucosidase for 4 hr (x), 8 hr (o), 24 hr (□), and 48 hr (Δ). The hydrolysis of oNPG (0.2 mM) was measured (o) in the presence of β-glucosidase at 37 °C.

reversed hydrolysis reaction of β- glucosidase from almond is approximately 4.0. In contrast, an optimum pH for the hydrolysis of oNPG (abbrviation : ortho Nitrophenyl β-D-Glucopyranoside) is approximately 5.5, which is in accordance with the value reported in the literature (Dale et al., 1986). The difference of the optimum pH between the hydrolysis reaction and synthesis reaction may be caused by the difference of the substrate concentration. Furthermore, it should be noted that the equilibrium concentration of disaccharides was consistent for all the pH ranges examined.

Effect of Concentration The relationship between the yield of disaccharides and initial glucose concentration is shown in Figure 4. The total yield of disaccharides increased almost linearly with the increase of the initial glucose concentration. The concentration of water was calculated from an equation suggested by Adachi et al (1984) and was accompanied with the glucose concentration in Figure 4. Even a 90% glucose solution, the concentration of water is 22M in contrast to 5M of glucose.

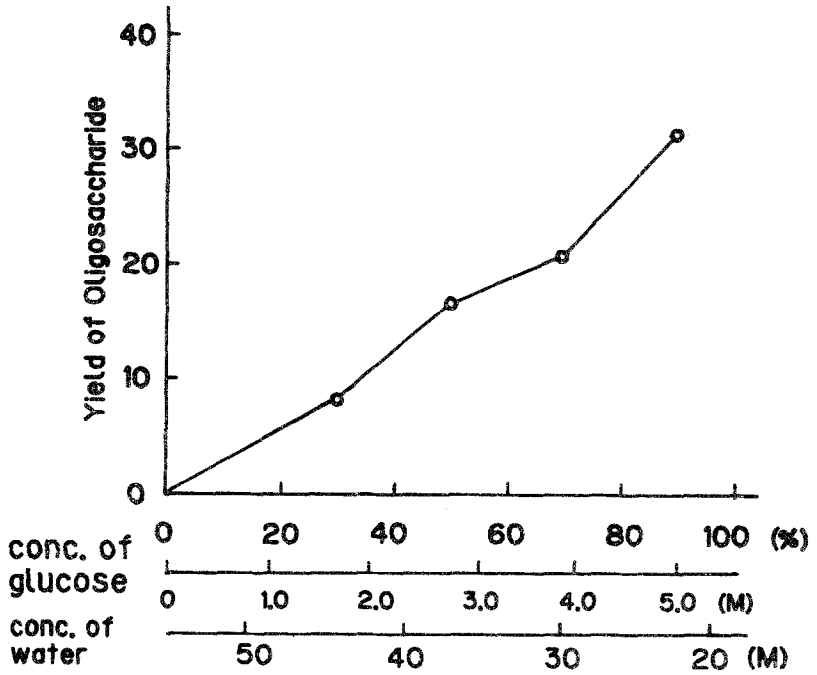

Figure 4 : Effects of concentration of glucose and water on the formation of disaccharides. The glucose solution of various concentration was incubated in the presence of 110 units/ml β-glucosidase at 37°C for 24hr. For the concentration of water, see text.

Although it can be shown that the glucose concentration is the most important factor to increase the yield of oligosaccharides, the glucose concentration of 90% is maximum on account of the high viscosity of the solution.

Temperature Dependency In order to examine the effect of temperature on the yield of disaccharides, the 90% glucose solution in the acetate buffer (pH 4.0) was incubated for 24 hr at 37°C and 55°C, respectively. The yield of each reaction is summarized in Table I. Although the composition of the disaccharides was independent on temperature, the total yield of disaccharides increased about 10% as the reaction temperature was raised from 37°C to 55°C. However, 55°C was the maximum temperature because the enzyme lost its activity at higher temperature than 55°C.

Table I. Temperature dependence of the yield of disaccharides.

Temp.	L	S + C	G	total
37°C	3.6%	8.0%	19.7%	31.3%
55°C	4.2	11.5	25.7	41.4

In conclusion, the oligosaccharide synthesis reaction by use of β-glucosidase proceeded fastest at pH 4.0. The yield of disaccharides increased as the initial concetnration of glucose increased and as the reaction temperature was raised.

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