Note

Conversion of Sucrose into Cellobiose Using Sucrose Phosphorylase, Xylose Isomerase and Cellobiose Phosphorylase

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Cellobiose was synthesized from sucrose by coupling reactions of sucrose phosphorylase, xylose isomerase and cellobiose phosphorylase in the presence of a catalytic amount of inorganic phosphate (P_i). When a reaction mixture (10 ml) containing 200 mM sucrose (684 mg), 20 mM P_i , 0.26 U/ml sucrose phosphorylase, 0.034 U/ml xylose isomerase and 0.21 U/ml cellobiose phosphorylase in 50 mM imidazol-HCl buffer (pH 7.0) was incubated at 37°C, the concentration of cellobiose in the reaction mixture reached 147 mM after 100 hr (yield 73%). Finally, 286 mg of crystalline β -cellobiose was recovered from the reaction mixture by charcoal column chromatography and crystallization.

Cellobiose has attracted increasing attention as one of the soluble dietary fibers and/or biologically functional food materials. Numerous reports have been published on the production of cellobiose from cellulose by chemical or enzymatic methods.¹⁻⁵⁾ It is well known that cellulose is acetylated into cellobioseoctaacetate by acetic anhydride and sulfuric acid with a high yield.¹⁾ Partial acid hydrolysis of cellulose is another one of the most intensively studied methods.^{2,3)} However, these chemical methods are not suitable for production of cellobiose when the cellobiose is planned to be used as a food material.

Utilization of cellulase is another popular method to produce cellobiose from cellulose.⁴⁻⁷⁾ A group of cellulase, called *exo*-cellobiohydrolase [EC 3.2.1.91], has been found in several microorganisms and produces cellobiose with a high yield from cellulose.⁴⁻⁷⁾ However, no enzyme preparation that is powerful enough to be used for industrial production of cellobiose is available at present.

In this paper, a completely new approach is proposed to produce cellobiose. The new method starts from sucrose, which is converted by successive actions of three enzymes, sucrose phosphorylase [EC 2.4.1.8], xylose isomerase [EC 5.3.1.5] and cellobiose phosphorylase [EC 2.4.1.20], into cellobiose with a high yield.

Sucrose phosphorylase partially purified from *Leuconostoc mesenteroides* was purchased from Sigma Chemical Co. (St. Louis, USA). Xylose isomerase, often called glucose isomerase, was purchased from Kanto Chemicals (Tokyo, Japan) as dry cells of *Streptomyces*. One gram of the dried cells was suspended in 10 ml of 50 mM imidazol-HCl buffer (pH 7.0) and disrupted by sonication. Ammonium sulfate was added to the resultant supernatant until the solution became 80% saturation and the precipitate formed was recovered and used as a xylose isomerase preparation. Cellobiose phosphorylase was partially purified from *Cellvibrio gilvus* cells using DEAE-Toyopearl 650S column chromatography in accordance with the method

Fig. 1. Time profile of the reaction by the three enzymes.

■, concentration of cellobiose; ●, concentration of sucrose. The reaction was carried out in a 2 ml reaction mixture containing 100 mM sucrose, 10 mM P_i , sucrose phosphorylase (0.26 U/ml), xylose isomerase (0.034 U/ml) and cellobiose phosphorylase (0.21 U/ml) in 50 mM imidazol-HCl buffer (pH 7.0) at 37°C. The sugar component of the reaction mixture was analyzed periodically by HPLC.

described by Kitaoka *et al.*⁸⁾

One unit of sucrose or cellobiose phosphorylase was defined as the amount of the enzyme which produced 1 μ mol G-1-P per min from 10 mM sucrose or cellobiose, respectively, and 10 mM P_i at 37°C in 50 mM imidazol-HCl buffer (pH 7.0). That of xylose isomerase was defined as the amount of the enzyme which produced 1 μ mol glucose per min from 10 mM fructose in the same condition. Amount of G-1-P was measured by the phosphoglucomutase-glucose-6-phosphate dehydrogenase system.⁹⁾ That of glucose was done by the glucose oxidase-peroxidase system with mutarotase¹⁰⁾ using Glucose C Test Wako (Wako Pure Chemicals, Osaka, Japan). The amounts of sucrose and cellobiose were measured by HPLC equipped with RI detector using LiChrosorb NH_2 column (Cica-Merck, Japan) and 65% acetonitrile as a solvent.

The new method consists of three enzymatic reactions as following reaction formulae.

At first, sucrose is phosphorolyzed into fructose and G-1-P by sucrose phosphorylase in (1). The resultant fructose is converted to glucose by xylose isomerase (glucose isomerase) in (2). Then glucose and G-1-P are condensed into cellobiose with release of P_i by cellobiose phosphorylase in (3). When the three reaction formulae are summed up, P_i , fructose, glucose and G-1-P disappear from the resultant formula as (4). Formula (4) suggests that sucrose can be stoichiometrically converted into cellobiose in a single reaction mixture containing the three enzymes and a catalytic amount of P_i . Furthermore, the equilibriums of the reactions of sucrose phosphorylase¹¹⁾ and cellobiose phosphorylase⁸⁾ are known to be favorable to phosphorolysis and synthesis, respectively. That of xylose isomerase is known to be approximately unity.¹²⁾ Considering these facts, the equilibrium of formula (4) is thought to be favorable to cellobiose. This suggests strongly the possibility that cellobiose is produced effectively in a reaction mixture containing the three enzymes, sucrose and a catalytic amount of P_i .

The possibility was examined as shown in Fig. 1. The sucrose content in the reaction mixture decreased from 100 to 17 mM after 100 hr with a concomitant increase in the cellobiose content from 0 to 76 mM. This result indicates that cellobiose can be produced from sucrose with a high yield (76%), just as anticipated.

In the next experiment, 10 ml reaction mixture containing 200 mM sucrose (684 mg), 20 mM P_i , and the same concentrations of the three enzymes as described above, was incubated at 37°C. After 100 hr, when the cellobiose concentration was 147 mM (yield 73%), the reaction mixture was boiled to inactivate the enzymatic activities and then it was treated with invertase (from bakers yeast, grade V, Sigma) until remaining sucrose disappeared. This solution was charged to a charcoal (Cargon GCP, Toyo Cargon, Tokyo) column (15 $\times$ 300 mm), and after monosaccharides were washed out with 2% ethanol, the cellobiose was eluted with 25% ethanol and crystallized to yield 286 mg of crystalline β -cellobiose. The obtained compound was identified by gas-liquid chromatography. The yield of the final preparation

was 42%.

It was found that sucrose was converted into cellobiose with 75% yield by the successive actions of the three enzymes. The loss in the yield that occurred during the isolation procedures may be minimized by scale up and optimization of each procedure. The efficiency of the method will be improved by using immobilized preparations of the three enzymes.

Sucrose is one of the most inexpensive carbohydrates commercially available. Xylose isomerase is produced industrially and is commercially available. Recently, sucrose phosphorylase gene of *L. mesenteroides* was cloned in *Escherichia coli* and a hyper production of this enzyme by a recombinant *E. coli* cells was reported.¹³⁾ Though cellobiose phosphorylase is not commercially available at present, the industrial application of this enzyme will be possible in near future. When this enzyme becomes available in an industrial scale, a gate to the industrial production of cellobiose will be opened.

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シュクロースホスホリラーゼ,
キシロースイソメラーゼおよび
セロビオースホスホリラーゼを
用いたシュクロースの
セロビオースへの変換

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セロビオースを, シュクロースを原料として触媒量のリン酸存在下でシュクロースホスホリラーゼ, キシロースイソメラーゼおよびセロビオースホスホリラーゼを同時に作用させることにより合成した. 10 ml の 50 mM イミダゾール-塩酸緩衝液 (pH 7.0) 中で, 200 mM シュクロース (684 mg) を 20 mM リン酸存在下, 0.26 U/ml シュクロースホスホリラーゼ, 0.034 U/ml キシロースイソメラーゼ, 0.21 U/ml セロビオースホスホリラーゼにより 37°C で反応させたところ, 反応 100 時間後に反応混液中のセロビオース濃度は 147 mM (収率 73%) に達した. 反応混液を活性炭カラムクロマトグラフィーにかけ, セロビオースを単離・結晶化させたところ, 286 mg の β -セロビオースの結晶が得られた (収率 42%).