Reaction pattern of glycogen branching enzyme from *Synechocystis sp. PCC 6803* on selected corn starches

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옥수수 전분 기질에 따른 GBE의 반응 특성

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## Objectives

- To determine the action patterns of glycogen branching enzyme (GBE) on the selected starches.

- To produce structure-modified starch products by GBE treatment.

- To determine the change in molecular structure by analyzing molecular weight and branch-chain distribution after GBE treatment.

## Materials and Methods

- Enzyme and reaction condition

GBE  $(10^{-1}-10^{-3} \text{ U/mL})$  was reacted at 30°C for 6 hrs with various substrates (potato amylose, normal corn starch, waxy corn starch, and amylomaize  $\mathbb{VI}$ ).

- Molecular structure determination by HPSEC and HPAEC

The molecular size distribution of GBE-treated starch was determined by high-performance size-exclusion chromatography (HPSEC, Summit HPLC system, Dionex, CA, USA). The reactant was chromatographically separated by Shodex SB-806 HQ and Shodex SB-804 HQ columns (Showa, Denko, Japan). The mobile phase was deionized water at the flow rate of 1.0 mL/min.

Branch-chain distribution of GBE-treated starch was determined by high-performance anion-exchange chromatography (HPAEC, DX-300 series chromatography system, Dionex, CA, USA). The product treated with isoamylase was separated by CarboPac<sup>™</sup> PA1 column (0.4×250 mm, Dionex, CA, USA). The two mobile phases were 160 mM NaOH and 160 mM NaOH with 600 mM NaOAc at the flow rate of 1.0 mL/min.

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## Results

- Molecular size distribution of GBE-treated starch

Molecular structure of GBE-treated products was determined by using HPSEC and HPAEC techniques. In case of waxy and normal corn starches, slight decreases in AP molecular size were observed with 0.001–U GBE treatment while drastic degradation of AP with 0.01– and 0.1–U GBE. Peak molecular weights ( $M_p$ )of these GBE-treated products were of  $5.3\times10^4$  (waxy) and  $6.0\times10^4$ (normal), respectively. Greater proportion of AM in amylomaize VII and potato AM resulted in somewhat different Mw distribution after GBE treatment. Clear bimordal Mw distribution were detected from amylomaize VII and potato AM. When amylomaize VII and potato AM were treated with GBE, intermediate size products ( $M_p$ =1.11x10<sup>6</sup> and 8.2x10<sup>5</sup>, respectively) between AP and AM were obviously detected at 0.1–U GBE treatment. As a result of HPSEC analysis, AP molecules completely degraded to the relatively narrow Mw range of  $5.3-9.4\times10^4$  while GBE synthesized much larger branched molecules from AM substrate.

- Branch-chain distribution of GBE-treated starch

In order to determine the branch-chain distributions of GBE-treated products, HPAEC analysis was performed after isoamylolysis. As the GBE dosage increased, the most abundant branch chain product was maltohexaose from all the GBE-reacted products. Overall *weight-* and *number-*average DPs (DP<sub>w</sub> and DP<sub>n</sub>) decreased from all the samples along with increasing GBE units. At the highest level (0.1 U/mL) of GBE treatment, the decreases in DP<sub>w</sub> were clear from 16.5 to 10.5 (amylomaize VII); from 15.2 to 10.8 (normal); from 14.2 to 11.8 (waxy). Consequently, GBE-treated products resulted in highly branched structure and greater water solubility compared with their native counterparts.



Figure 1. Molecular size distribution of GBE-treated starches

Figure 2. Branch-chain distribution of GBE-treated starches