Enzymatic synthesis of trehalose 6-phosphate from maltose

Yodai Taguchi1, Wataru Saburi1, Ryozo Imai2, Haruhide Mori1

1 Laboratory of Biochemistry, Research Faculty of Agriculture, Hokkaido University,
2 Institute of Agrobiological Sciences, National Agriculture and Food Research Organization.

E-mail address of presenter: yodai@chem.agr.hokudai.ac.jp
Key Words: Trehalose 6-phosphate, Trehalose 6-phosphate phosphorylase, Maltose, Phosphorylase coupling reaction

Trehalose 6-phosphate (Tre6P: α-D-GlcP-(1→1)-α-D-GlcP6P), the intermediate of trehalose biosynthesis, has profound regulatory functions on plant metabolism, growth, and development. However, functional analysis of Tre6P is limited, because no efficient production system of Tre6P has been established thus far. Tre6P synthesis through fermentation by yeast has been published, but the yield of Tre6P was low. Therefore, in this study, we attempt to develop the efficient enzymatic synthesis of Tre6P using trehalose 6-phosphate phosphorylase (TrePP) from Lactococcus lactis sp. lactis which reversibly catalyzes the phosphorolysis of Tre6P to generate β-glucose 1-phosphate (β-Glc1P) and glucose 6-phosphate (Glc6P).

Recombinant L. lactis TrePP was produced in Escherichia coli BL21 (DE3). From the cells harvested from 1 L of culture fluid, 19 mg of purified recombinant TrePP was obtained by nickel chelating column chromatography. This enzyme produced 85 mM Tre6P from 100 mM β-Glc1P and 100 mM Glc6P after the reaction at 30°C for 36 h (yield, 85%). To reduce the cost for the Tre6P production, first, β-Glc1P was replaced by maltose as an alternative, but maltose phosphorylase (MP) was added. Phosphorolysis of maltose provided β-Glc1P, the substrate for the Tre6P production by TrePP (Fig. 1). Through the coupling reaction for 28 h, 65 mM Tre6P was synthesized from 100 mM maltose and 100 mM Glc6P in the presence of 20 mM inorganic phosphate (Pi) (yield, 65%). Second, Glc6P was excluded as a starting material, but β-phosphoglucomutase (β-PGM) was supplemented to convert β-Glc1P to Glc6P (Fig. 1). It enabled to use maltose as a sole carbohydrate source. Reactions at various Pi and maltose concentrations were tested, and the highest concentration of Tre6P (34 mM) was obtained from 100 mM maltose with 50 mM Pi in 72-h reaction (yield, 68%), however, 15 mM Glc6P was formed. By reducing Pi to 20 mM, β-Glc1P and Glc6P formation was suppressed (β-Glc1P: 0.2 mM, Glc6P: 2.3 mM) and accumulation of 20 mM Tre6P was observed after 28-h reaction (yield, 39%). Produced Tre6P can be easily separated from residual maltose and glucose by electrodialysis, but not from the other sugar phosphates. Compared with the synthesis from β-Glc1P and Glc6P, residual sugar phosphates can be reduced in the reaction from maltose. For this reason, this enzymatic synthesis of Tre6P from maltose is effective in terms of purification.

Figure 1. Reaction scheme of Tre6P synthesis from maltose by TrePP, MP and β-PGM