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Sugar Transport and Utilization in *Corynebacterium glutamicum*: Analysis of the PTS and Heterologous Expression of Fructokinase

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Corynebacterium glutamicum is widely used for the industrial production of amino acids, especially glutamic acid and lysine, thus extensive genetic improvements have been made to overproduce various amino acids. Since the recent release of the complete genome sequence of *C. glutamicum* ATCC 13032, extensive genome-wide analyses and strain improvements have also been conducted on this amino acid producer. However, despite numerous investigations of sugar metabolism and its regulation in *C. glutamicum*, relatively few studies have been conducted on the sugar transport systems and their regulation on a molecular level.

Studies on the sugar transport of sucrose, fructose and glucose are very important, as the major carbon source for industrial lysine fermentation is molasses, which contains these three sugars. Several biochemical and physiological studies on transport have revealed that many sugars are taken up by *C. glutamicum* via the phosphotransferase system (PTS). As one of the strategies for improving amino acid production, the PTS may be a good target because it is the first step of sugar metabolism, and PTS-associated global carbon regulation also seems to play an important role in sugar metabolism. However, only three PTS genes encoding enzyme I (EI), glucose enzyme II (EII), and β-glucoside EII have so far been characterized from *C. glutamicum*.

In this study, we found that *C. glutamicum* has four EII genes of the PTS in its genome encoding transporters for sucrose (*ptsS*), glucose (*ptsG*), fructose (*ptsF*), and an unidentified EII from the public genome sequence data. To analyze the function of these EII genes, they were inactivated via homologous recombination and the resulting mutants characterized for sugar utilization. Whereas the sucrose EII was the only transport system for sucrose in *C. glutamicum*, fructose and glucose were each transported by a second transporter in addition to their corresponding EII. Northern blot analysis showed that the expression of *ptsS* was induced by sucrose or fructose, while *ptsG* was expressed constitutively, regardless of the sugar, in contrast to *E. coli* or *B. subtilis*.

Sucrose 6-phosphate hydrolase hydrolyzes the sucrose 6-phosphate formed by the sucrose PTS into glucose 6-phosphate and fructose. The wild-type *C. glutamicum* accumulated only a small amount of

fructose on the sucrose minimal medium very early in the exponential growth phase, and this was rapidly consumed during the rest of the exponential growth phase. In contrast, the *ptsF* mutant accumulated fructose in the culture broth during growth on the sucrose minimal medium, indicating that the intracellular fructose derived from sucrose 6-phosphate hydrolysis was excreted into the culture broth. Since the fructose produced by sucrose hydrolysis is converted to fructose 1-phosphate via efflux followed by import through fructose EII, this raised the idea of making a pathway for the direct phosphorylation of intracellular fructose using fructokinase without any fructose efflux. Modifying the fructose metabolic pathway from fructose EII to fructokinase could alter the route of metabolism. As no fructokinase gene exists in the *C. glutamicum* genome in contrast to most sucrose operons from other bacteria, the fructokinase gene from *Clostridium acetobutylicum* was expressed in *C. glutamicum.* The heterologous fructokinase gene was successfully expressed in *C. glutamicum*, thereby phosphorylating intracellular fructose directly without fructose efflux. The expression of fructokinase could also increase the flow to the pentose phosphate pathway (PPP) for the supply of anabolic reducing power on a sucrose medium, as this enzyme can directly phosphorylate intracellular fructose into fructose 6-P instead of fructose 1-P, which is formed by fructose EII. Therefore, changing the route of fructose entry into metabolism via fructokinase could be beneficial for lysine production during growth on sucrose due to an increased supply of NADPH. In addition, fructokinase uses ATP instead of PEP as a phosphoryl donor, thus fructokinase expression would seem to be a potential strategy for enhancing amino acid production. Lastly, possible global carbon regulation related to the PTS in *C. glutamicum* will also be discussed.

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