Regulating the biosynthesis of pyridoxal 5'-phosphate with riboswitch to enhance L-DOPA production by *Escherichia coli* whole-cell biotransformation

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May 5, 2020

Abstract

Pyridoxal 5'-phosphate (PLP) is an essential cofactor that participates in ~4% enzymatic activities cataloged by the Enzyme Commission. The intracellular level of PLP is usually lower than that demanded in industrial catalysis. To realize the self-supply of PLP cofactor in whole-cell biotransformation, the *de novo* ribose 5-phosphate (R5P)-dependent PLP synthesis pathway was constructed. The pdxST genes from *Bacillus subtilis* 168 were introduced into the tyrosine phenol-lyase (TPL)-overexpressing *Escherichia coli* BL21(DE3) strain. TPL and PdxST were co-expressed with a double-promoter or a compatible double-plasmid system. The 3,4-dihydroxyphenylacetate-L-alanine (L-DOPA) titer did not increase with the increase in the intracellular PLP concentration in these strains with TPL and PdxST co-expression. Therefore, it is necessary to optimize the intracellular PLP metabolism level so as to achieve a higher L-DOPA titer and avoid the formation of L-DOPA–PLP cyclic adducts. The *thi* riboswitch binds to PLP and forms a complex such that the ribosome cannot have access to the SD sequence. Therefore, this metabolite-sensing regulation system was applied to reduce the translation of mRNA. Riboswitch was introduced into pET–TPL–pdxST-2 to downregulate the expression of PdxST and biosynthesis of PLP at the translation level by sequestering the ribosome-binding site. As a result, the titer and productivity of L-DOPA using the strain BL21–TPLST–Ribo1 improved to 69.8 g/L and 13.96 g/L/h, respectively, with a catechol conversion of 95.9% and intracellular PLP accumulation of 24.8 μM.

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